

**STATE-OF-THE-ART PAPERS  
BIOLOGICAL SCIENCES**

National Academy of Science and Technology  
Republic of the Philippines  
1986

# **STATE-OF-THE-ART PAPERS BIOLOGICAL SCIENCES**



Published by  
**THE ACADEMY**  
Bicutan, Taguig, Metro Manila  
Philippines

National Academy of Science and Technology  
Bicutan, Taguig, Metro Manila  
Philippines

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ISBN 917-8538-01-1

Printed in  
THE REPUBLIC OF THE PHILIPPINES

## **GENERAL PREFACE**

The Division of Biological Sciences, National Academy of Science and Technology, Republic of the Philippines has sponsored this State-of-the-Art series so that those interested can learn about significant developments in the field of Biology in the country.

The features of the series include selected topics and wherever possible suggestions for further development and change.

Reader's comments will be appreciated.

Paulo C. Campos  
President, NAST

## **PREFACE**

A book of this length can only serve as an introduction to the very many ways in which the discipline of Biology can help enhance our knowledge in this country. The contents represent an attempt to indicate the scope of the field in selected areas.

In this short book we have tried to show some of the many ways in which R and D can be enhanced in the Biological Sciences and keep-up-to date on some of the facets of the discipline.

We hope that the Biological Sciences will continue to be seen as a living scientific discipline of interest and relevance to everyone.

Carmen C. Velasquez  
Chairman, Division of  
Biological Sciences, NAST

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## CONTENTS

	Page
Preface . . . . .	iii
Contributors . . . . .	iv
MAGDALENA C. CANTORIA. Chemical Plant Taxonomy . . . . .	1
<i>Discussion Papers:</i>	
MILDRED BALBIN-OLIVEROS. Chemotaxonomy in the Family Zingiberaceae . . . . .	25
BEATRICE Q. GUEVARA. Flavonoids as Systematic Markers . . . . .	27
WILLIAM G. PADOLINA. Distribution of Special Classes of Compounds in the Family Compositae . . . . .	29
JOVENTINO D. SORIANO. Efficient Seed Irradiation and Mutant Selection with Emphasis on Peanut and Sorghum . . . . .	35
JÓSE R. VELASCO and ZENaida N. SIERRA. Current Efforts in Plant Eco-Physiology . . . . .	45
CLARE R. BALTAZAR. Taxonomy of Philippine Diptera . . . . .	85
CARMEN C. VELASQUEZ. Fish Parasitology and Aquaculture Management in the Philippines . . . . .	105



# CHEMICAL PLANT TAXONOMY\*

Magdalena C. Cantoria\*\*

## Introduction

The concept that plants can be classified on the basis of their chemical constituents is not new. To pharmacists, the idea of grouping medicinal plants according to the chemical nature of their active constituents (alkaloids, glycosides, volatile oils, etc.) is accepted practice and can be found in the writings of herbalists of the fifteenth century who sometimes arranged their plants according to their medicinal properties (Gibbs, 1963, 1974; Trease and Evans, 1978). Early workers classified the algae into blue-green, green, brown, and red forms, primarily on the basis of the colors which they could see, but it turns out that these color differences are best understood in terms of the chemistry of the pigments concerned (Cronquist, 1973). At the beginning of the eighteenth century, botanists, notably Nere-miah Grew, James Petiver, and Rudolf Jacob Camerarius, recognized that some groups of plants which possessed certain therapeutic properties also coincided with the morphological arrangement then current as exemplified by members of the families Labiatae, Cruciferae, and Umbelliferae (Gibbs, 1963, 1974). A. P. de Candolle, in the early years of the nineteenth century, laid the foundations for the study of chemotaxonomy by his observations on such topics as the compatibility of grafts, susceptibility of groups of plants to insect attack, and bitterness in plants as criteria for taxonomic classification. By the beginning of the twentieth century, the distribution in plant species of such readily detectable groups of compounds as cyanogenic substances, tannins, alkaloids, and saponins had received considerable attention. The development of modern methods of isolation and characterization has made it possible to screen thousands of plant samples for their active constituents.

Although the use of chemical characters in taxonomy and systematics has a very long history, the emergence of the hybrid discipline *biochemical systematics* or *chemotaxonomy* as a distinctive and developed field of study dates back to the early sixties (Heywood, 1973a). It was marked by the publication of such works as Alston and Turner's pioneer text, *Biochemical Systematics* (1963), the

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\*Paper presented at the *State-of-the-Art* Seminar Series of the Biological Sciences Division, National Academy of Science and Technology, held at the NAST Conference Room on December 18, 1985.

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symposium edited by Swain, *Chemical Plant Taxonomy* (1963), and by the first volumes of Hegnauer's *Chemotaxonomie der Pflanzen* (1962-1965).

### Chemical Constituents as Taxonomic Characters

Classical taxonomists regard the various chemical constituents of plants as characters to be considered along with other features of the plant traditionally used in taxonomy (Trease and Evans, 1978). The main consideration of chemical data so far, in this respect, has been to ascertain how well, or otherwise, the distribution of chemical constituents fits in with the classical taxonomic schemes. Problems concerning evaluating characters are as equally applicable to chemical constituents as to other taxonomic characters and in many instances parallel trends emerge between classical taxonomic groupings and chemical constituents.

Generally, the principal constituents studied in relation to classification are those secondary metabolites (Table 1) which have an intermediate frequency of occurrence in the plant kingdom (Alston, 1966; Harborne, 1968; Trease and Evans, 1978). Obviously those plant constituents, such as the hexoses and other primary metabolites, which are of almost universal occurrence are of little interest for comparative studies and their metabolic pathways must have evolved early in the evolutionary scale. Similarly, compounds which are now known to occur in one species only, are of little interest unless biogenetically related to similar constituents of other plants.

Chemical characters offer the advantage over morphological ones in that they are often more precise and easily defined; on the other hand, with the exception of those characters which are rapidly detectable (pigments, odoriferous principles), it has rarely been possible to check their occurrence in thousands of plants as has been done for many morphologic features (Trease and Evans, 1978). Furthermore, when deciding on the relative abundance or absence of a particular constituent, account must be taken of the age and degree of development of the plant, the plant part used for analysis, and other factors causing variation in the amount of the constituent present (Hegnauer, 1977). Thus, the composition of the oil from the flowering peppermint top will be different from that obtained from the vegetative top, and orange oil from the rind of the fruit is different from the oil of orange flowers.

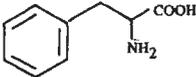
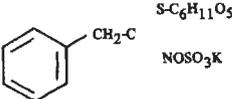
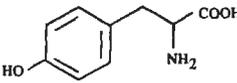
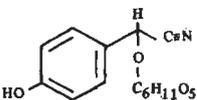
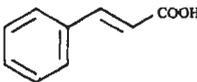
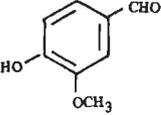
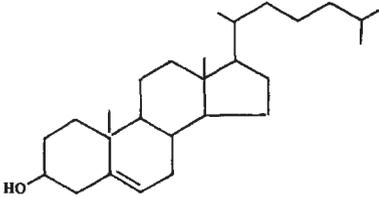
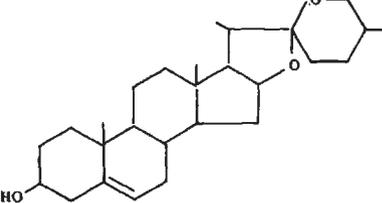
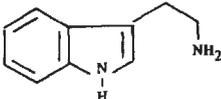
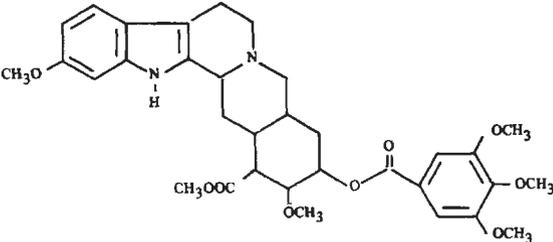
Many complex compounds arise in plants from quite simple building units of widespread occurrence but secondary modifications and rearrangements of the final structure may be more limited and therefore of interest to chemotaxonomists (Trease and Evans, 1978). Examples of such modifications are shown in Table 2.

The more thermodynamically feasible the reaction, the more likely it is to occur independently in unrelated plants. Phenols are readily hydroxylated, methylated, and glucosylated. From the phenolic acid, *p*-coumaric acid (Fig. 1), a wide

Table 1. Secondary metabolites of chemotaxonomic interest

<i>Chemical Group</i>	<i>Examples/Occurrence</i>	<i>Chemotaxonomic Interest</i>
1. Hydrocarbons of the alkane series	Components of cuticular waxes of leaves, stems, flowers, and pollen grains	Intraspecific variation in waxes; wax production genetically controlled.
2. Fatty acids	Components of seed oils and fats	A characteristic pattern shown in proportion of individual fatty acid relative to the total fatty acid mixture.
3. Glycosides	C-glycosides in Liliaceae (anthraquinone type) and in Leguminosae and 17 other families (flavonoid type); S-glycosides in Cruciferae, Capparidaceae, Moringaceae, Resedaceae	Usually considered in terms of their aglycones; of interest because of their distribution and biological activity.
4. Cyanogenic compounds	In more than 80 families of angiosperms, a few gymnosperms, several ferns, and some fungi	Of interest to taxonomy at the order, family, and species levels.
5. Phenolic compounds	Trihydroxy derivatives of benzoic and cinnamic acids; hydroxybenzoquinones and hydroxynaphthoquinones in Ranunculaceae and Plantaginaceae; flavonoids in Leguminosae, Compositae, Apocynaceae, and Gramineae; betacyanin and betaxanthin pigments in the Order Centrospermae	The distribution of particular phenolics of taxonomic value; biosynthetic pathways probably useful in phylogeny.
6. Terpenoids	Glycosides of triterpenes (saponins) in the dicotyledons; monoterpenes, diterpenes, and sesquiterpenes in higher plants; volatile oils in Labiatae, Myrtaeae, etc.	Great variety in chemical structure despite common origin from mevalonic acid; their distribution useful in taxonomy; the comparative chemistry of modern species of phylogenetic significance.
7. Alkaloids	Commonly found in the orders Centrospermae, Magnoliales, Ranunculales, Papaverales, Rosales, Rutales, Gentianales, Tubiflorae, and Campanulales	Alkaloid formation a taxonomic character, subject to the limitations of parallelism and divergence.

Table 2. Secondary modifications of common metabolites

Common Metabolites of Widespread Occurrence	Modified Compounds of Limited Occurrence
$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	$\text{CH}_3(\text{CH}_2)_7\underset{\text{C}}{\text{C}}(\text{CH}_2)_7\text{COOH}$
Oleic acid	Stercic acid in the Malvaceae and Sterculiaceae
	
Phenylalanine	Glucotropaeolin in <i>Tropaeolum majus</i> L. (Tropaeolaceae), Cruciferae
	
Tyrosine	Dhurrin in <i>Sorghum vulgare</i> Pers. (Gramineae)
	
Cinnamic acid	Vanillin in <i>Vanilla spp.</i> (Orchidaceae)
	
Cholesterol	Diosgenin in <i>Dioscorea spp.</i> (Dioscoreaceae), fenugreek (Leguminosae), <i>Trillium spp.</i> (Liliaceae)
	
Tryptamine	Reserpine in <i>Rauwolfia spp.</i> (Apocynaceae)

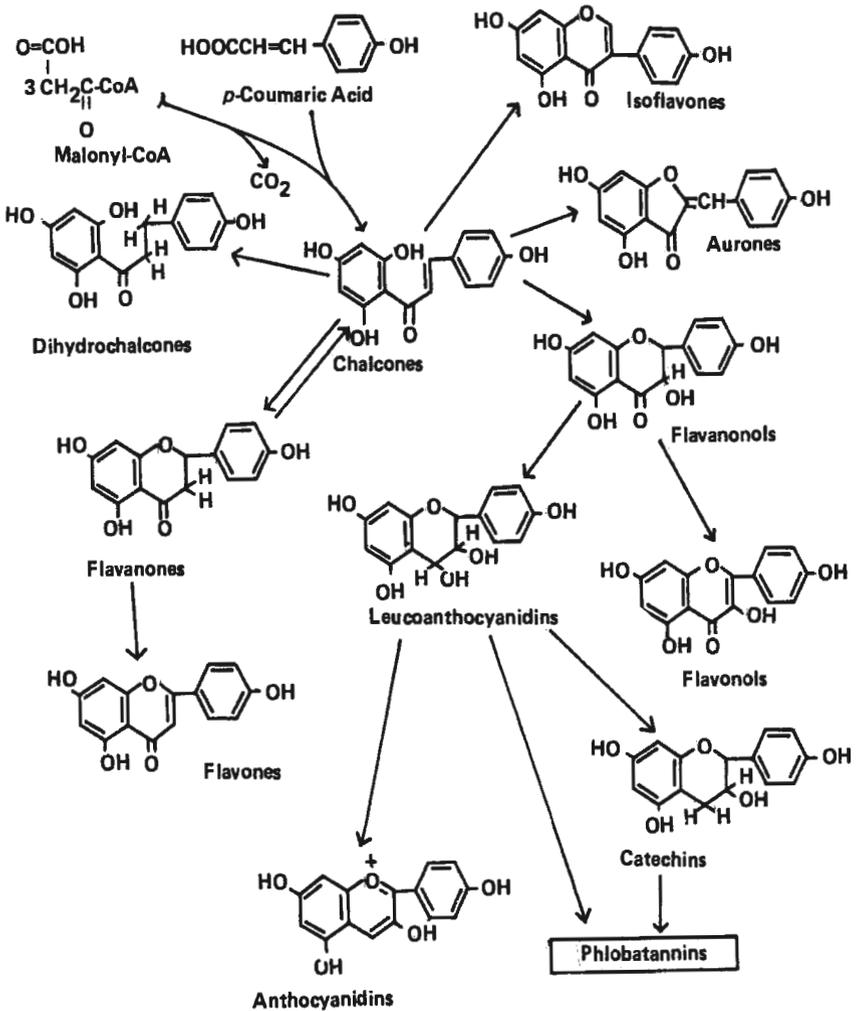


Fig. 1. From the phenolic acid, *p*-coumaric acid, a wide spectrum of flavonoids has evolved independently in many unrelated plants (Robinson, 1983).

spectrum of flavonoids has evolved independently in many unrelated plants (Bate-Smith, 1963; Robinson, 1983; Smith, 1976). Oxidations such as those involving the saturated C-ring of steroids, e.g., the cardenolides (Fig. 2), appear less probable on thermodynamic grounds and are found to be restricted in their occurrence in nature.

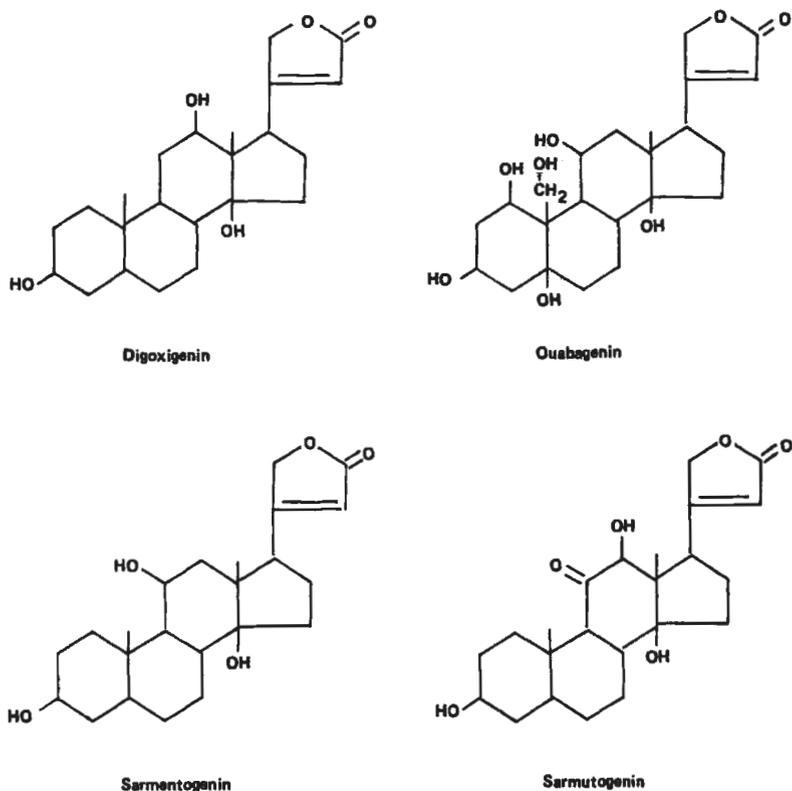


Fig. 2. Oxidations, such as those involving the saturated C-ring of steroids, are restricted in their occurrence in nature, e.g., the cardenolides: digoxigenin in *Digitalis lanata* Ehrh. (Scrophulariaceae), ouabagenin in *Strophanthus gratus* (Wall. & Hook.) Baill. (Apocynaceae), and sarmentogenin and sarmutogenin in *S. sarmentosus*.

### Divergence and Convergence

During evolution, it is possible that related groups of plants may give rise to morphologically dissimilar descendants (Erdtman, 1963), thus belying their true affinity. Conversely, unrelated groups of plants may give rise to morphologically similar ones. The former is called *divergence* or *diversification* and the latter, *convergence* or *parallelism*. These phenomena can apply to chemical as well as to morphological characters and they can cause considerable taxonomic difficulties (Alston and Turner, 1963); Erdtman, 1963; Hegnauer, 1977; Heywood, 1966; Takhtajan, 1973; Trease and Evans, 1978).

As a result of divergent development, related plants may produce chemically unlike products with a common biogenetic origin (homologous dissimilarity, homologous characters; Fig. 3). These plants probably contain similar enzyme systems and the compounds which they produce may therefore indicate that a relationship exists between them (Erdtman, 1963; Hegnauer, 1977).

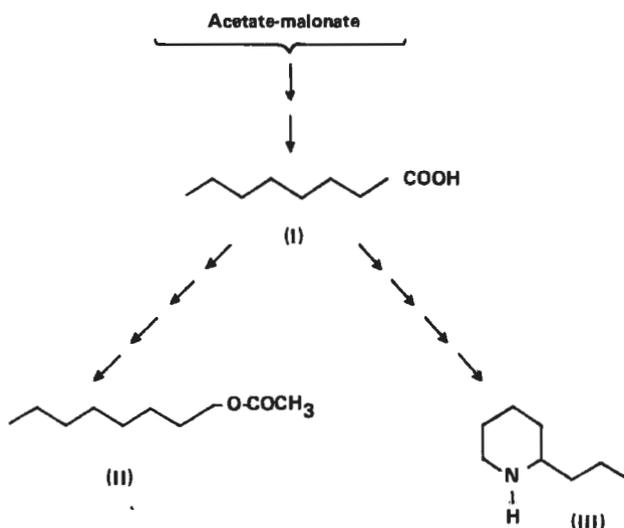


Fig. 3. An example of homologous dissimilarity (divergence or diversification). Two chemically different plant constituents have a similar biogenetic origin. (II) and (III) are homologous characters which may (but need not) indicate true relationships. (I) Octanoic acid (caprylic acid); (II) Octanol-1 acetate, a main constituent of the volatile oil of the fruit of *Pastinaca sativa* L. (Umbelliferae); and (III) Coniine, the main alkaloid of the fruit of *Conium maculatum* L. (Umbelliferae) (Hegnauer, 1977).

Frequently, chemical similarity (analogous similarity, analogous characters; Fig. 4) may arise from convergent evolution, that is, the same compound or very similar compounds may originate along different pathways in unrelated plants (Alston and Turner, 1963); Hegnauer, 1977; Takhtajan, 1973). On the other hand, other obviously unrelated groups of plants may elaborate the same metabolic end product along the same biosynthetic pathway when the pathway is relatively simple (separated by only a few and very common steps from an essential metabolite). Such chemically similar compounds (Fig. 5) should not be used in establishing taxonomic relationships.

Consideration of these problems due to divergence and convergence makes it clear that biosynthetic pathways and patterns of occurrence of constituents, as distinct from the mere presence or absence of particular constituents, are of fundamental importance in detecting relationships between groups of plants.

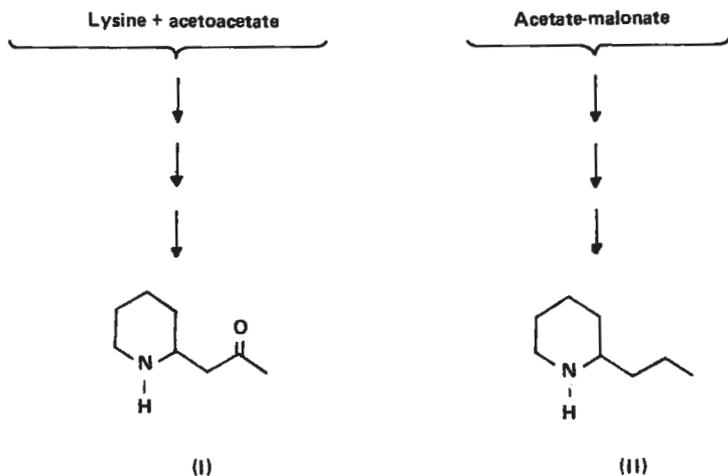


Fig. 4. An example of analogous similarity (convergence or parallelism). Two chemically very similar compounds originate along different pathways. (I) and (II) are analogous characters which should not be used in establishing taxonomic relationships: (I) Isopelletierine in Crassulaceae, Punicaceae, and Solanaceae; and (II) Coniine in *Conium maculatum* L. (Umbelliferae) (Hegnauer, 1977).

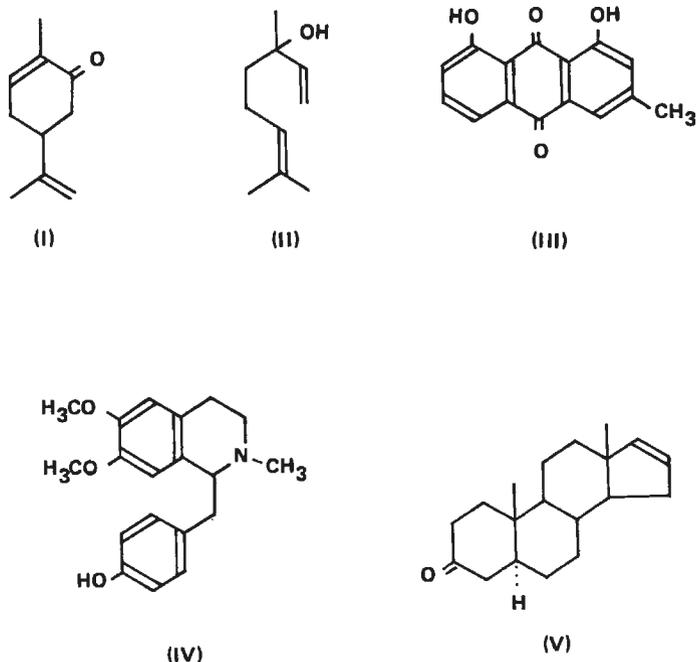


Fig. 5. Some examples of chemical convergences in nature: (I) Carvone, a monoterpene ketone occurring in algae, gymnosperms, dicotyledons, and monocotyledons; (II) Linalool, one of the most widespread monoterpene essential oil constituents of vascular plants (free and esterified); (III) Chrysophanol, an acetogenic anthraquinone occurring in fungi and several families of dicotyledons and monocotyledons; (IV) Armepavine, a benzylisoquinoline-type alkaloid occurring in *Nelumbo nucifera* Gaertn. (Nymphaeaceae), *Papaver spp.* (Papaveraceae), *Euonymus europaeus* L. (Celastraceae), *Discaria spp.* (Rhamnaceae), and *Zanthoxylum spp.* (Rutaceae); and (V) 5 $\alpha$ -Androsta-16-en-3-one, the boar pheromone steroid which is known from pig, man, and two umbelliferous root vegetables (celery and parsnip) (Hegnauer, 1977).

### Evolutionary and Taxonomic Information From Proteins and Nucleic Acids

The amount of evolutionary and taxonomic information highly increases from nonpolymeric secondary constituents to proteins and nucleic acids (Takh-tajan, 1973). There is, therefore, a definite tendency towards the study of these two major groups of polymeric molecules rather than to less informational end products of metabolic processes or secondary constituents. While numerous cases of convergence are known in the evolution of secondary compounds, it seems highly unlikely that proteins of the same primary structure can be produced by evolutionary convergence.

It is generally agreed that comparative studies on primary structures of proteins, that is, amino acid sequence determination, provide the most valuable information on evolutionary classification (Cronquist, 1973; Smith, 1976; Takhtajan, 1973; Watts, 1970). Available data indicate that this approach can be expected to correlate reasonably well with the more traditional ones. Amino acid sequences can be evaluated independently of all other taxonomic characters and can provide an independent check on the system. Unfortunately, the technical difficulties in investigating the amino acid sequence of proteins considerably limit the choice of proteins for comparative studies.

Though proteins are but functional translations of operational units of DNA, nucleic acid chemistry seems less reliable as a source of evolutionary insight and taxonomic study (Takhtajan, 1973). While a comparative study of the degree of pairing between complementary strands of nucleic acids of different organisms may be eventually very useful, very many difficulties both in the technique and interpretation limit the value of molecular hybridization.

### **Toxic Metabolites**

Some toxic metabolites, like alkaloids and phenolic compounds, are produced in trace quantities by many plants but considerable accumulation may occur in only a few genera (Trease and Evans, 1978). These plants have acquired the ability to store such toxic metabolites. Hegnauer (1962-1973) considers this another important aspect of comparative phytochemistry because the ability to store, as well as synthesize, toxic metabolites constitutes another taxonomic character for consideration.

The role of allelopathy, or the control of growth, health, behavior, and population biology of organisms by chemicals produced by other organisms, on the evolution of vegetation has been recognized (Gibbs, 1974; Smith, 1976). Substances with allelopathic effects include phenolic compounds, flavonoids, terpenoids, steroids, alkaloids, and organic cyanides. Some ecological aspects of terpenoids are shown in Fig. 6 (Hegnauer, 1977).

The phytoalexins, which include phenolic compounds, are present in the epidermis of the protected organ as a first defense and are produced in quantity in the deeper tissues surrounding a fungal penetration through the epidermis as a second defense (Gibbs, 1974; Smith, 1976). An example is pisatin, an isoflavone derivative, which is accumulated by pea tissue in the presence of a wide range of fungal species.

### **Chemical and Biochemical Methods Applied to Taxonomy**

Rapid advances in comparative phytochemistry have become possible with the development of separation techniques in chromatography and spectroscopy.

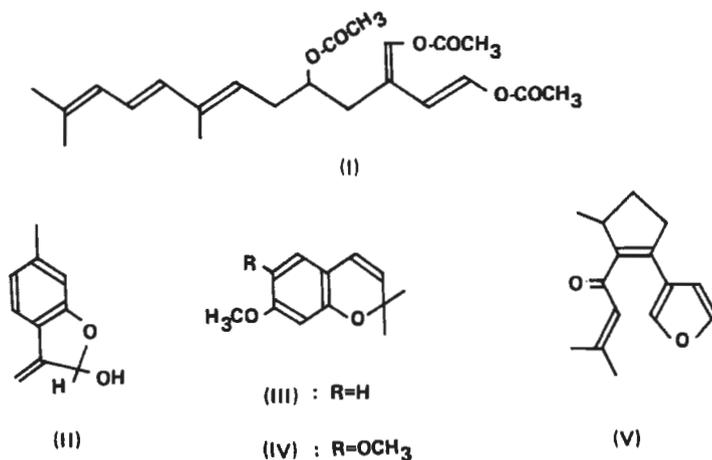


Fig. 6. Some ecologically important steam-volatile plant constituents: (I) Rhipocephalin from the green alga *Rhipocephalus phoenix*; induces pronounced feeding avoidance behavior in a species of herbivorous fish; (II) A nematocidal thymol derivative occurring in several species of *Helenium* (Compositae); (IV) Precocene 2 (ageratochromene) and its 6-desmethoxy derivative (III) (precocene 1) in the volatile oil of *Ageratum houstonianum*; disturb the metamorphosis of heteropterous insects by suppression of the production of juvenile hormone; have a sterilizing effect (ovicidal action) on adult females of other insect groups; and (V) Dehydromyodesmone, a highly toxic sesquiterpenoid from the volatile oil of a chemical race of *Myosporum deserti* Hegnauer, 1977).

These have led to the isolation and characterization of new compounds, the screening of thousands of plant species, and the elucidation of biosynthetic pathways. Two biochemical procedures of importance in chemical plant taxonomy are plant serology and DNA hybridization (Kohne, 1968; Smith, 1976; Trease and Evans, 1978).

Animals can be immunized by successive treatment with a protein-containing plant extract which acts as the antigen (Smith, 1976; Trease and Evans, 1978). Antibodies are produced by the animal and its blood serum, treated with the antigenic plant extract, will produce a characteristic precipitin reaction. Related antigenic extracts from other plants will react similarly, although to various extents, with the antiserum; extracts from unrelated plants which do not contain the same antigenic proteins will not give the same reaction. Improved serological techniques employ gel diffusion methods using agar plates. In a number of cases there appears to be considerable correlation between serotaxonomic and classical taxonomic schemes.

The technique of DNA hybridization may be considered one of the most fundamental available for purposes of comparative phytochemistry because it amounts to a comparison of DNA molecules from different sources (Kohne, 1968; Smith, 1976; Trease and Evans, 1978). It is possible for a double helical DNA molecule to be separated into its two complementary component strands. These single strands, under suitable conditions, can come together again and combine to form a complete molecule (reassociated DNA). However, the component strands will be different from those originally forming the pair. If radioactive single strand DNA in low concentration is mixed, under associating conditions, with nonlabeled single strand DNA in high concentration and then the unpaired single strands are removed by suitable means, the new reassociated DNA can be collected and its activity will indicate to what extent hybridization has taken place between the species. This assumes that the low concentration of radioactive single stranded DNA employed prevents it from combining with itself. Collisions will occur only with the nonradioactive DNA which will also rapidly reassociate with itself. In this way, species, varieties, etc. of organisms can be compared on the basis of the extent of their DNA hybridization. DNA hybridization has been applied mainly to viral, bacterial, and mammalian DNA's. Studies have been reported on the application of the technique to comparing nucleotide sequence in the DNA's of some legumes and cereal grains.

### Chemotaxonomy of Volatile Oils

Hegnauer (1977, 1978) defines essential (volatile) oils as complex mixtures of odorous and steam-volatile compounds which are accumulated by plants in the subcuticular space of glandular hairs, in cell organelles (oil bodies of Hepaticae), in idioblasts (oil cells), in excretory canals (oil ducts), in excretory cavities (oil glands), or, in rare instances, in heartwoods (e.g., sandalwood). The presence or absence of volatile oils, regardless of their composition, represents a very valuable taxonomic character. This character is a combination of secretory structures (anatomical aspect) and production of steam-volatile secretions (chemical aspects).

In the Order Tubiflorae, glandular hairs are very widespread but generally they do not produce appreciable amounts of volatile oils (Hegnauer, 1977, 1978). Many members of the closely related families Verbenaceae and Labiatae are highly aromatic and have a high yield of volatile oil. In both families, however, there are groups of very aromatic genera and groups of scarcely aromatic genera. In Labiatae, there is a clear-cut phytochemical distinction (Table 3). The group of aromatic plants does not produce iridoid glycosides; at the most, some nonglycosylated iridoid compounds like nepetalactone, myodesertal, and myodesertin may be present in the volatile oils. The nonaromatic group is characterized by iridoid glycosides like ajugol, galiridoside, harpagide, lamiol, metittoside, and others. The two chemical groups of Labiatae coincide with a previously proposed classification for the family.

Table 3. Phytochemical groups in the Family Labiatae (=Lamiaceae) (Hegnauer, 1978)

<u>Branch A</u>	<u>Branch B</u>
Mainly low in oil Iridoid glycosides present	Mainly high in oil Iridoid glycosides never present
Prostantheroideae Ajugoideae Scutellarioideae Stachyoideae	Saturejoideae with 13 tribes (including Nepeteae, Saturejeae, Rosmarineae, Lavanduleae, Monardeae, Salviae)

Iridoid compounds are biologically very active substances (Hegnauer, 1977). Insecticidal and insect-repellant activities have been attributed to steam-volatile iridoids such as nepetalactone (V) and iridomyrmecin and isoiridomyrmecin (VI) (Fig. 7). Many iridoid glycosides are known to be toxic to insects and vertebrates and to have more or less pronounced antibiotic properties.

The families listed by Engler under Tubiflorae were later reclassified by Dahlgren (Hegnauer, 1977) under the two superorders, Solananae and Lamianae, and the other Lamiales. In most cases, the rearrangements agree with the chemistry of the respective taxa.

### Chemotaxonomy of Volatile Oil Constituents

A functional approach to chemical characters is rewarding; the more we know about their biogenesis and functions, the more we should be alert to possible convergences (Hegnauer, 1977). Volatile oils are considered secondary metabolites of plants (or natural products). Usually secondary metabolites are not directly involved in growth and reproduction, but rather in fitness for life. Volatile oils, like other secondary metabolites, are more involved in ecology than in the physiology of plants.

The bulk of well known constituents of volatile oils occurs in many taxonomically unrelated taxa [examples I and II of Fig. 5] (Hegnauer, 1977). Such compounds are taxonomically useful at the generic level. Moreover, they can be very useful in biosystematic studies which aim at an understanding of intraspecific differentiation and speciation. The study of chemical races represents one aspect of experimental plant taxonomy. Plants belonging to a chemical race have similar phenotypes but different genotypes and as such are identical in external appearance but different in their chemical constituents. Chemical races are chemically distinct populations, not merely deviating genotypes in a local population. The former always originate from the latter, but the differentiation of

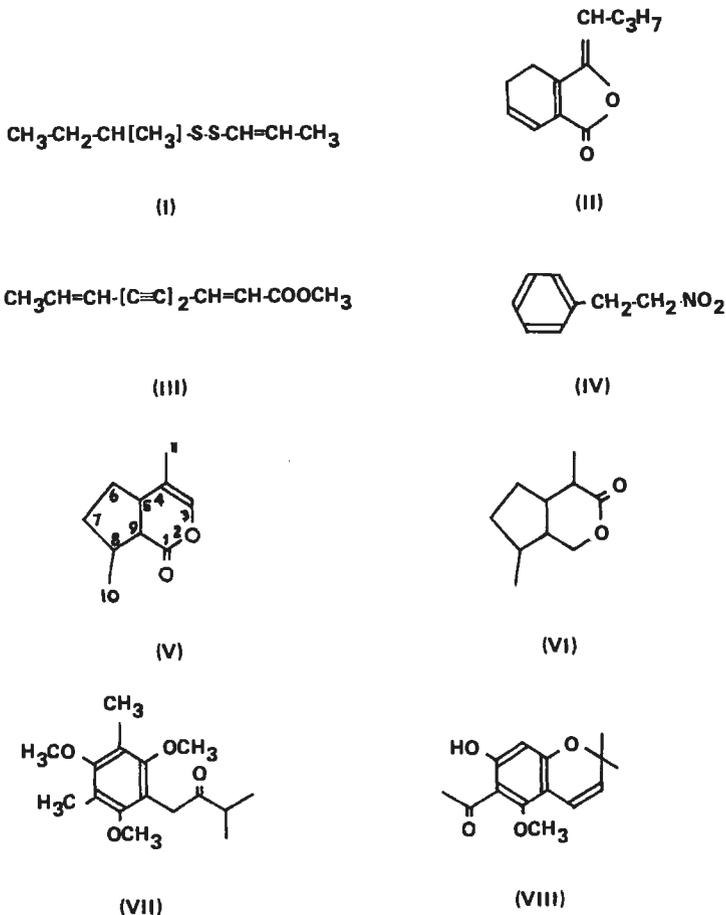


Fig. 7. Examples of more or less taxon-specific volatile oil constituents: (I) *sec*-Butylpropyl disulfide, a component of the oleoresin of a new species of *Ferula* (Umbelliferae); (II) Ligustilide, a phthalide occurring in the volatile oils of species of *Angelica*, *Cnidium*, *Conioselinum*, *Levisticum*, and *Meum* (Umbelliferae), probably an acetogenic compound; (III) Matricaria ester, an ester of a C-10 polyacetylenic carboxylic acid, occurring in the volatile oils of many Compositae; (IV) Nitrophenylethane, a cinnamon-scented constituent of the volatile oils of some Lauraceae; (V) Nepetalactone, major constituent of the oil of catnip (Labiatae); (VI) Iridomyrmecin and isoiridomyrmecin (iridolactones) which occur in anal glands of ant species of the genus *Iridomyrmex*; (VII) Torquatone, a phloroglucinol derivative in some species of *Eucalyptus* (Myrtaceae); and (VIII) Evodionol, a phloracetophenone derived 2, 2-dimethylchromene in some species of *Ovodia*, *Medicosma*, and *Melicope* (Rutaceae) (Hegnauer, 1977).

a race is a long-lasting process. On the basis of chromosome counts and chemical analysis of monoterpenes in the volatile oils from several populations, Lawrence (1978) established the existence of chemical races or chemotypes in several *Mentha* species and hybrids. Examples are chemotypes in *M. longifolia* (L.) Huds.<sup>1</sup>, *M. suaveolens* Ehrh., and *M. x cordifolia* Opiz (Table 4).

Some oil constituents can readily be termed taxon-specific because they seem to occur in only a few species (examples I and IV, Fig. 7) or because they express biochemical tendencies within genera, tribes, families, orders, or classes [examples: Fig. 6, (II) and (V); Fig. 7, (II), (III), (V & VI), (VII), and (VIII)] (Hegnauer, 1977). Such tendencies may sometimes indicate phylogenetic relationships between higher taxa such as genera and families.

Biosynthetic relationships have been shown for *Mentha* species (Fig. 8) (Reit-sema, 1958). Studies have been reported on the metabolic interconversions of the 3-oxygenated monoterpenes in peppermint (Fig. 9) (Burbott and Loomis, 1967; Loomis and Croteau, 1980) and the biosynthesis of two series of oxygenated compounds in *Mentha* species (Fig. 10) (Loomis, 1967).

A fruitful application of volatile oil constituents to scientific plant classification requires a knowledge of the causes of their variation qualitatively and quantitatively. In *Mentha* species grown in the U.P. Diliman campus in Quezon City, the following plant factors have been observed to affect oil yield and composition: age of the plant, development stage of the plant, genetics, and existence of chemical races (Cantoria, 1977, 1980, 1985). Monthly and seasonal variations and modifications by daylengths have also been noted.

Some results obtained which are of significance to taxonomy are the identification of Philippine yerba buena as *Mentha cordifolia* Opiz (not *M. arvensis* L. as previously thought), the identification of piperitenone oxide (3-oxygenated monoterpene) as the principal constituent of its oil, the identification of carvone (2-oxygenated monoterpene) as the chief constituent of volatile oil of the American chemotype, and the recognition of a hairy strain of *M. cordifolia* with carvone as the chief constituent of its oil.

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<sup>1</sup>For various reasons, the scientific names of plants are revised by taxonomists. To avoid confusion, the name of the plant and the name of the botanist responsible for it must be mentioned. *Illicium verum* Hook. f. (Chinese star anise) is the same plant as *I. anisatum* Lour. but the former name is valid. *I. religiosum* Sieb. & Zucc. (Japanese star anise) is also known as *I. anisatum* L. & Gaertn. The danger in confusing one for the other lies in the fact that the Japanese fruit is very poisonous while the Chinese fruit is used as a flavoring for food and pharmaceuticals. Care should be taken in the spelling of Latin names because misspelling and subsequent "misprints" can lead to much confusion.

Table 4. Chemotypes in two *Mentha* species and one natural hybrid (Lawrence, 1978)A. Chemotypes in *Mentha longifolia* (L.) Huds. based on fifteen populations

- Chemotype 1. Oils rich in *cis*- and *trans*-piperitone oxide and piperitenone
- Chemotype 2. Oils rich in carvone/*trans*-carvyl acetate and limonene
- Chemotype 3. Oils rich in linalool

B. Chemotypes in *M. suaveolens* Ehrh. (*M. rotundifolia* auct. non (L.) Huds.) based on twenty populations

- Chemotype 1. Oils rich in 1, 2-epoxidized, 3-substituted compounds (piperitenone oxide/piperitone oxide type)
- Chemotype 2. Oils containing at 1(6) reduced, 2-substituted compounds (dihydrocarvone type)
- Chemotype 3. Oils containing at 1(6) unreduced 2-substituted compounds (carvone type)

C. Chemotypes in *M. x cordifolia* Opiz based on eleven populations

- Chemotype 1. Oils rich in carvone and limonene with lower quantities of other reduced 2-substituted compounds
- Chemotype 2. Oils containing piperitenone and *cis*- and *trans*-piperitone oxide

### Chemistry in Plant Classification

It is clear from literature that chemical and biochemical data may be applied to systematics just like many other characters. The goal is to develop a system of classification based upon the maximum correlation of attributes. Fig. 11 is a taxonomic flow chart showing how information from various sources, such as morphology, anatomy, ecology, physiology, genetics, chemistry, and biochemistry can be integrated to improve existing plant classification and to add to knowledge of phylogeny or evolutionary relationships. In the case of chemical evidence, the chemists or biochemists furnish chemical facts which are then available for the taxonomists to consider. Future research calls for closer cooperation, indicating the need for dialogues between taxonomists and chemists in order to explore theoretical and intellectual aspects of the subject, develop a basic rationale, integrate biological and chemical findings, and make a critical analysis of existing work.

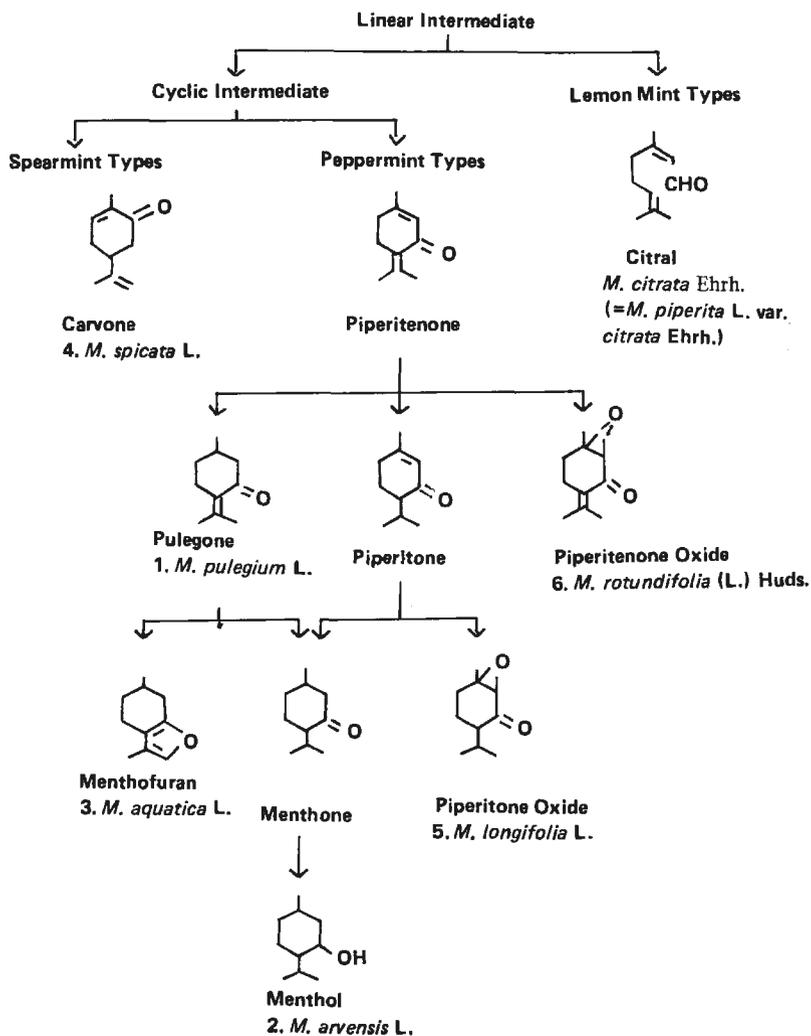


Fig. 8. Biosynthetic relationships of six *Mentha* species and one variety (after Reitsemá, 1958).

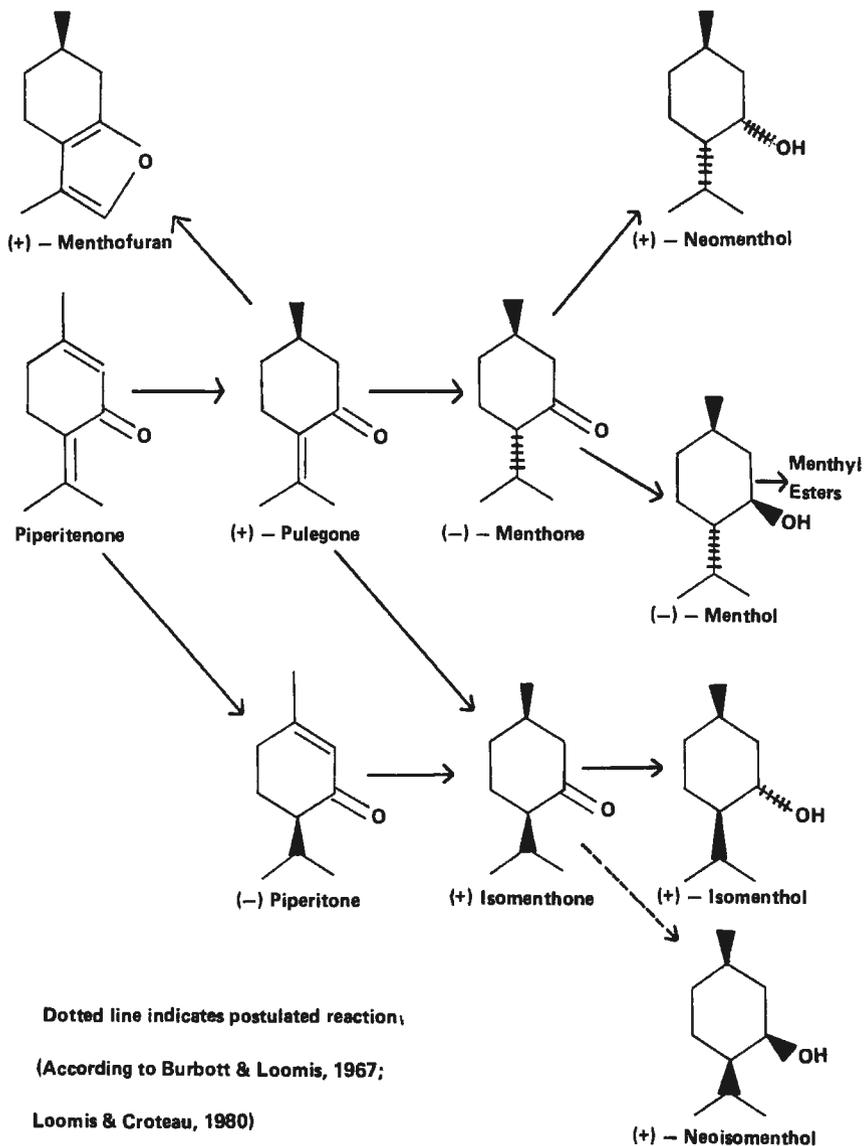


Fig. 9. Metabolic interconversions of the 3-oxygenated monocyclic monoterpenes in peppermint (Burbott and Loomis, 1967). Each of the reactions shown by solid arrows has been demonstrated in cell-free systems from peppermint and several of the enzymes have been partially purified (Loomis and Croteau, 1980).

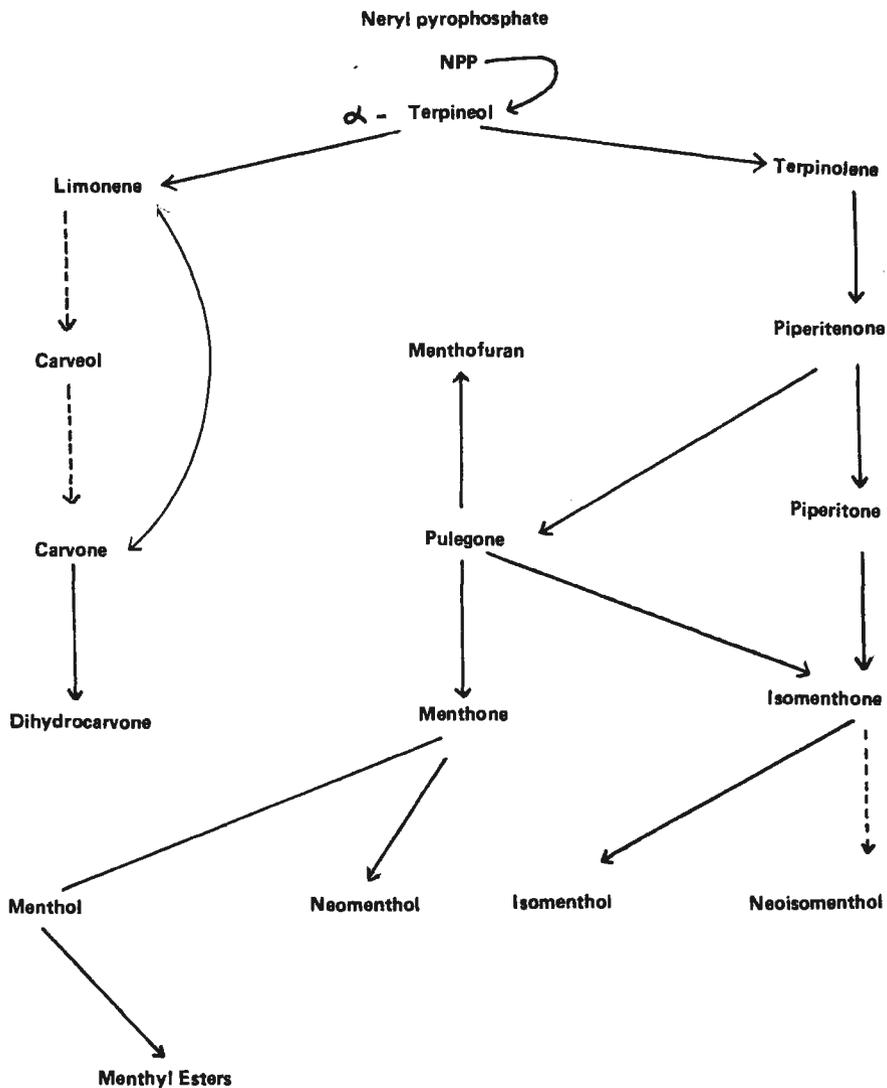


Fig. 10. Biosynthesis of two series of oxygenated compounds in *Mentha* species (Loomis, 1967). Reactions starting from limonene yield that 2-oxygenated (carvone) series. The terpinolene-piperitenone transformation leads to the 3-oxygenated (pulegone) series. (See also Fig. 9)

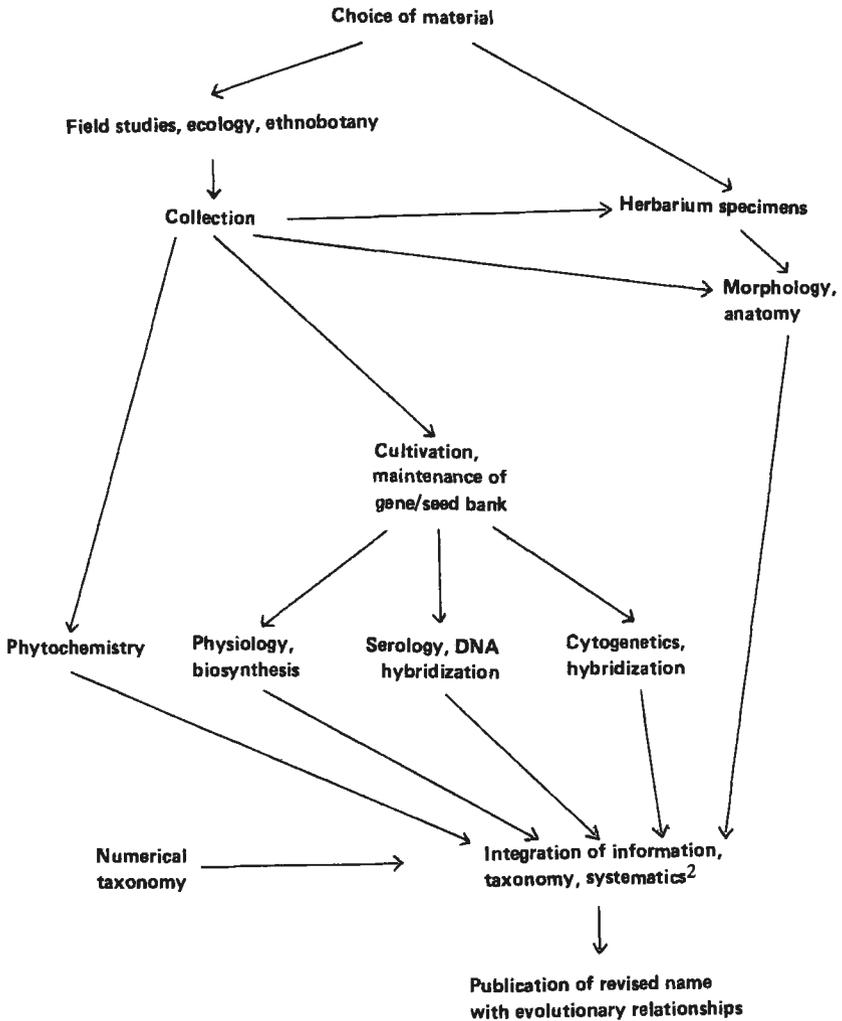


Fig. 11. Proposed flow chart of information for plant systematics (adapted from Smith, 1976).

<sup>2</sup>Systematics, in its broadest sense, is concerned with the scientific study of the diversity and differentiation of organisms and the relationships that exist between them (Heywood, 1973b). It embraces comparative phylogenetic, evolutionary, phenetic, morphological, traditional, classical, etc., approaches. Taxonomy is restricted to that part of systematics which deals with the study of classification, its bases, principles, procedures, and rules.

### Summary

1. No single source of information can substitute for the integrative approach to plant classification based on the correlation and synthesis of evidences from all available sources of knowledge.

2. Without the wide basis of comparative morphology, the chemical approach alone cannot introduce any fundamental changes into the present-day system of classification.

3. Chemical data have been considered mainly to ascertain how well, or otherwise, the distribution of chemical constituents fits in with the classical taxonomic schemes and to add confirmatory evidence to plant classification based on morphological and other characters.

4. Plant constituents of chemotaxonomic interest are the secondary metabolites with an intermediate frequency of occurrence in the plant kingdom.

5. Comparative studies of biochemical pathways and the recognition of biogenetic relationships can provide valuable information for evolutionary classification.

6. A growing tendency toward the study of proteins and nucleic acids yields more evolutionary and taxonomic information.

7. The ability of some plants to synthesize and store toxic metabolites constitutes another taxonomic character for consideration.

8. The application of chemical and biochemical techniques to plant taxonomy lies in the isolation and characterization of plant constituents, elucidation of biosynthetic pathways, serology, and DNA hybridization.

9. Chemical studies on the volatile oils of some *Mentha* species grown in the U.P. Diliman campus, supported by physiological studies, have yielded some results of taxonomic value.

10. The use of chemical and biochemical data in systematics necessitates the holding of dialogues between taxonomists and chemists to explore theoretical and intellectual aspects of the subject, develop a basic rationale, integrate biological and chemical findings, and to make a critical analysis of existing work.

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### Acknowledgments

The author acknowledges the inspiration provided by Dr. Carmen C. Velasquez, Academician, and Dr. Domingo A. Madulid for the concept of this paper. The kindness of Prof. Dr. Robert Hegnauer, internationally recognized leader in the field of plant chemotaxonomy, formerly of the Laboratorium voor Experimentele Plantensystematiek, Leiden, Netherlands, in sending reprints of his publications is gratefully acknowledged. The author also wishes to acknowledge the invaluable assistance extended by Mrs. Lilian V. Cuanang, UP College of Pharmacy Librarian; Mrs. Leonor Gregorio, UPLB University Librarian; Mrs. Minda Licuanan, UPD Science Reading Room Librarian; Miss Estela R. Ellado, research assistant; and the Secretariat of the National Academy of Science and Technology during the preparation of this paper.

The author takes this opportunity to thank the discussants at the Seminar: Dr. Domingo A. Madulid, Dr. Romualdo M. del Rosario, Dr. Benito C. Tan, Dr. William G. Padolina, Dr. Mildred B. Oliveros, and Prof. Beatriz Q. Guevara. Their enthusiastic participation was very encouraging and it is hoped that the National Academy of Science and Technology will be able to sponsor continuing dialogues between botanists and chemists in the area of chemical plant taxonomy in the future.



## CHEMOTAXONOMY IN THE FAMILY ZINGIBERACEAE\*

Mildred Balbin-Oliveros\*\*

The importance of chemotaxonomy has become increasingly obvious with our growing knowledge of the chemistry of natural products. It has played a very significant role in the classification of plants where classical biological methods have brought about confusion and disagreement among taxonomists. This can be traced to its use of chemical characters which are more precise than biological characters.

In the order Scitamineae, which is composed of the families Musaceae, Zingiberaceae, Marantaceae, Cannaceae, and Lowiaceae, a number of chemical characters have been found to be of taxonomic importance. Among these is the ability to produce volatile oils. This chemical character is used as a taxonomic marker for the Family Zingiberaceae because it has been found to be restricted to this family where secretory cells are extremely widespread (Hegnauer, 1977). This same chemical character is being used by some taxonomists in their proposal to split the Family Zingiberaceae into Zingiberaceae and Costaceae. The absence of volatile oils, in addition to the presence of steroid saponins, like diosgenin, a sapogenin in Costoideae, are the arguments being presented to support this proposal to exclude Costoideae from the Family Zingiberaceae (Hegnauer, 1977).

The presence of flavonoids with unsubstituted  $\beta$ -rings in the Family Zingiberaceae is another chemical character noted by Bate-Smith in 1963, Harborne in 1966, and Gibbs in 1974. Its usefulness as a taxonomic marker lies in the fact that flavonoids are actively concerned in the cellular metabolic processes. Thus, any particular flavonoid constituent can be relied on to be present in more or less constant amounts, in the same tissue of the same species for as long as the plant is grown under normal physiological conditions.

Examples of flavonoids with unsubstituted  $\beta$ -rings in the Family Zingiberaceae are: alpinetin in *Alpinia* and *Kaempferia*, pinostrobin in *Kaempferia*, alpinone, 5-hydroxy-7-methoxy-3-acetoxy flavanone, galangin, galangin-3-methyl ether, and galangin-7-methyl ether in *Alpinia* (Gibbs, 1974).

The similarity in flavonoid patterns of monocots and dicots was also noted by Harborne in 1966. He reported that the presence of a certain flavone, flavonol-4 methyl ether in both *Alpinia officinarum* of the Family Zingiberaceae and in *Tamarix* sp. of the Family Tamaricaceae shows a remarkable chemical link between very disparate families.

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\*Discussion paper on *Chemical Plant Taxonomy*.

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In Gibbs' chemical classification of the Order Scitamineae, he noted the occurrence of starch, silica cells, phenolic acids, and tannins in all the families; the presence of raphides in the Musaceae and Lowiaceae. He also found that Scitamineae is not an alkaloid-rich order.

In 1978, Trease and Evans reported the taxonomic value of starch grain forms in the Family Zingiberaceae. This is exemplified by the sack-shaped, beaked starch grains of ginger and *Curcuma*.

Chemotaxonomists working on Scitamineae encountered difficulties in the chemical characterization of individual families. All are in agreement that more information on the chemistry of the order is necessary to provide an adequate basis for its chemotaxonomic classification.

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## FLAVONOIDS AS SYSTEMATIC MARKERS\*

Beatrice Q. Guevara\*\*

Thin-layer chromatography has made possible the speedy separation of plant constituents into characteristic repeatable patterns called fingerprints. Similar fingerprint patterns have been found among plant species that are related as their fingerprints are reflections of their metabolic pathways.

Among the secondary plant metabolites studied for their fingerprints are the phenolics which include the flavonoids, the alkaloids, and plant proteins. Flavonoids are well known to be useful as taxonomic markers in many plants, particularly at the generic and specific levels, where patterns sometimes show useful correlations with morphology.

Like human fingerprints, chemical fingerprints of plant extracts are distinctive among closely related species showing some pattern similarities. Although alkaloid TLC patterns are not always reliable, phenolic compounds like the flavonoids are more reliable for fingerprinting. However, to be useful, chemical fingerprinting should be used in conjunction with standard chemical methods involving the isolation and characterization of plant constituents.

Flavonoids are phenolic plant pigments considered as secondary metabolites. They have the C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> carbon skeleton and occur either as free compounds or as glycosides. They may be hydroxylated or methoxylated at the flavone nucleus. More recently, acylated flavonoid O-glycosides have been reported.

Flavonoids may be separated and identified by thin-layer chromatography (TLC), visualized in daylight under UV light, exposed to ammonia vapors, and again examined under UV and daylight.

Flowers, pollens, leaves, roots – fresh or dried – may be used. The fresh plant parts may be macerated in acetone and petroleum ether and layered over water. The petroleum layer may be examined for lipophilic coloring materials while the aqueous layer, for glycosidic and polar aglycones.

Serial extraction is usually conducted for dried plant material using solvents of increasing polarity – petroleum ether, chloroform, diethyl ether, acetone, and finally methyl alcohol. The monophenolics are found in the chloroform layer and the aglycone and some glycosides in the ether layer while most of the other glycosides are in the acetone and methanol extracts.

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Each of the concentrated plant extracts is monitored by TLC. Silica gel G layer is best for the less polar compounds while polyamide layers, for free phenol groups and their glycosides. Cellulose layers are used for separating the more polar aglycones and those with many glycosidic units. To develop the chromatograms, various solvents, like butanol-acetic acid-water, have been used.

Colored spots are readily visible under daylight. Colorless spots which may be viewed on plates coated with fluorescent indicators, either fluoresce or quench light under UV light. Spraying the plate with  $\text{NH}_3$  may change the characteristic fluorescence under UV and daylight. The use of chelating salts of iron and aluminum also gives characteristic intensely-colored spots on polyphenols and flavone compounds. Most phenolics yield intensely orange and violet compounds by coupling with diazonium salt like Fast B Blue salt.

Documentation is done by a comparison of the  $R_f$  values, as well as the color of the spots observed when the extracts are chromatographed on the same plate.

The author's experience in chemical fingerprinting to ascertain the identity of two similar *Ipomoea* seeds was more on the alkaloids. To the experienced botanists, the differences between the two black seeds may be obvious, but fine distinctive marks may elude the chemists. TLC fingerprints of the chloroform extracts of the two seeds, visualized with Dragendorff's reagent showed very distinctive differences.

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## DISTRIBUTION OF SPECIAL CLASSES OF COMPOUNDS IN THE FAMILY COMPOSITAE\*

William G. Padolina\*\*

An excellent review of the chemotaxonomy of the Family Compositae (=Asteraceae) was made by Herout (1970). In that review, it was noted that the Compositae are a phylogenetically relatively recently-evolved family and rich in compounds of secondary metabolism.

While many secondary metabolites abound in Compositae – volatile oils, flavonoids, alkaloids, phenolic acids, other terpenoids, and polyacetylenic compounds – it has been felt that the distribution of a special group of terpenoids, the sesquiterpene lactones, and the polyacetylenes provide an interesting picture of the relationships of the tribes within the family.

### Sesquiterpene Lactones

Sesquiterpene lactones are highly oxidized sesquiterpenes characterized by the presence of the  $\gamma$ -lactone. The lactone moiety is believed to have been derived from the isopropyl groups of the original farnesene-type precursor. Besides the presence of this lactone, which has introduced at least two oxygen-atoms to the carbon skeleton, various other oxygenated functionalities have been found in the sesquiterpene skeletal system. Hydroxyl groups, esters, epoxides, transannular ethers, and even another lactone group are present in many sesquiterpene lactones.

While these compounds have been found mainly in Compositae, other plant families like Umbelliferae and Magnoliaceae have also indicated the presence of sesquiterpene lactones (Herout, 1970). Herz (1970) notes that while these compounds are not unique to Compositae, they appear to be characteristic secondary metabolites of the family or certain subgroups thereof.

A schematic representation of the proposed biogenetic scheme for sesquiterpene lactones is given in Fig. 1. The scheme represents the relationships of the basic types of carbon skeletons of the lactones. The detailed biogenetic pathways for these are, no doubt, under enzymic control but published reports in this regard are very scanty.

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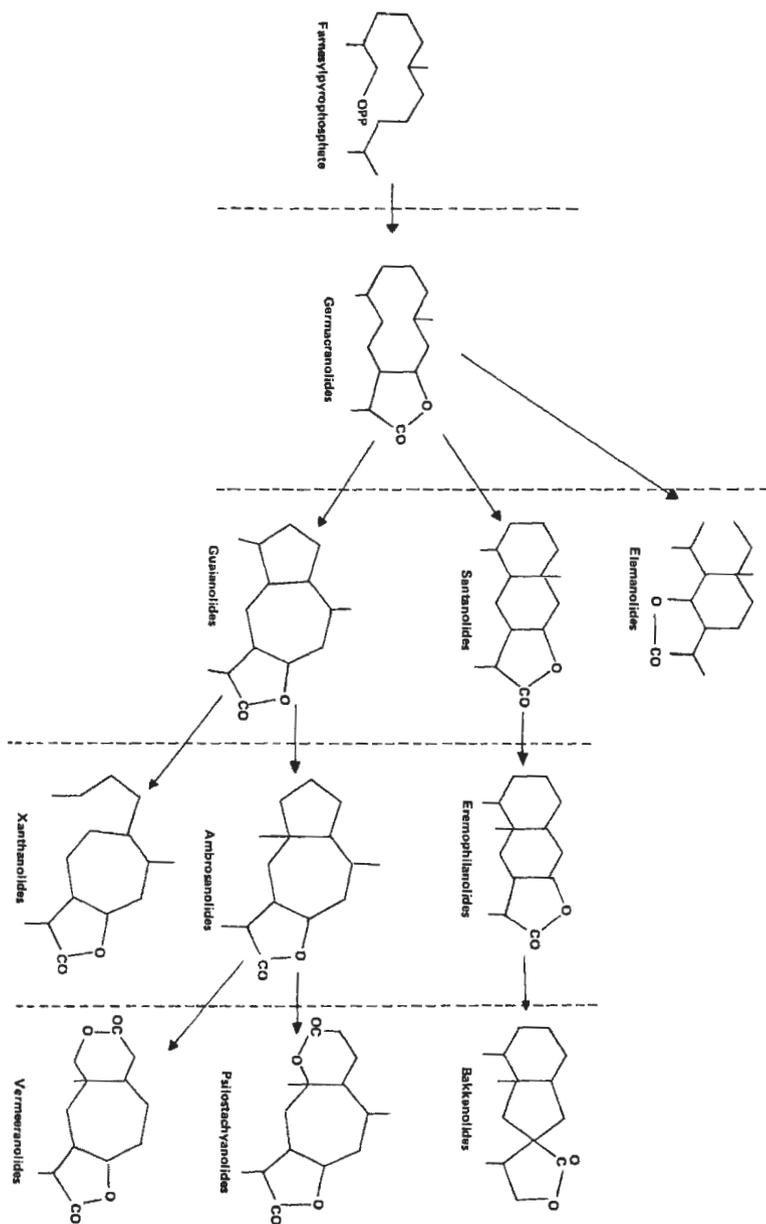


Fig. 1. Schematic representation of the biogenesis of different classes of sesquiterpene lactones.

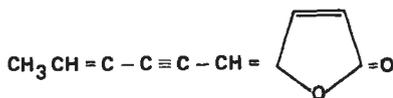
The distribution of sesquiterpene lactones in the individual tribes of Compositae is given in Table 1 as summarized by Herout (1970). The distribution of sesquiterpene lactones by species is also presented by Yoshioka *et al.* (1973).

Generally, the sesquiterpene lactones in Compositae are derived from a biogenetic series which are typical of the tribes. The simplest lactone is the monocyclic germacranolide skeleton and the more elaborate skeletal systems are derived from the germacranes.

Some of the tribes are characterized by very specialized skeletal systems. In the tribe Vernoniaeae, the germacranolides predominate. In the case of the tribe Cynareae (Cardueae) most of the species studied do not show the presence of germacranolides, but in several genera, guaianolides are produced but no santanolides are observed. Anthemideae produce germacranolides but no pseudoguaianolides have been found. This kind of analysis could be extended to other tribes in Compositae (Herout, 1970; Yoshioka *et al.*, 1973). Suffice it to say that on the basis of a similar evaluation of the tribes of Compositae, the Senecioneae remains separated for it is the only tribe containing the eremophilanolide lactones. Herout (1970) presented a scheme of evolutionary relationships of the tribe of Compositae based upon the biogenetic relationship of sesquiterpene lactones in correlation with the botanical characters.

### Polyacetylenes

Polyacetylenes are compounds which contain the carbon-carbon triple bond in an unbranched aliphatic moiety attached to a great variety of functional groups. A few examples are given below:



Matricaria lactone



Dehydromatricaria acid

Polyacetylenes have been found in fungi and a number of higher plants.

Biogenesis of polyacetylenes proceeds in most cases via oleic acid and other polyunsaturated acids (Luckner, 1972). The double bonds are converted by dehydrogenation to triple bonds and the chains may be shortened by conversion to vinyl compounds or by  $\alpha$  or  $\beta$ -oxidation. Other functional groups like the formation of phenyl rings, thioethers and furan derivatives have also been observed with polyacetylenes (Luckner, 1972).

The regularities in structural variation which characterize polyacetylenes and the considerable number of such compounds reported in Compositae make the

use of these compounds as phylogenetic markers appropriate (Gottlieb, 1982). The distribution of polyacetylenes in the different tribes is given in Table 2.

From the distribution of these compounds, Gottlieb (1982) suggests that a low relative oxidation and the C<sub>18</sub> chain are indicative of primitive traits. There is a gradation of more highly oxidized and shorter chains for the more advanced families. Using this as a basis, Gottlieb (1982) further notes that the polyacetylene distribution data support the suggestion by other systematists that the Senecioneae, Cynareae, and Heliantheae are the primitive tribes. In the case of the other tribes, the trends are not that clear yet but tentative clusters may be made.

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Table 1. The frequency of occurrence of sesquiterpene lactones (Herout, 1970)

Tribe	Number of plant species investigated	Number of lactones of different types per tribe									
		GE <sup>x</sup>	EL	SA	ER	BA	GU	XA	AM	PS	VE
Vernonieae	5	4	3	—	—	—	—	—	—	—	—
Eupatorieae	5	6	1	—	—	—	7	—	1	—	—
(Astereae)	—	—	—	—	—	—	—	—	—	—	—
Inuleae	11	1	—	12	—	—	9	3	1	—	1
Heliantheae	56	2	—	3	—	—	5	1	10	—	—
(Heleniinae)	—	7	—	6	—	—	9	—	52	—	7
Ambrosieae	38	10	—	10	—	—	7	9	26	4	—
Anthemideae	57	13	—	35	—	—	19	—	—	—	—
Senecioneae	16	—	—	—	34	6	—	—	7	—	—
(Calenduleae)	—	—	—	—	—	—	—	—	—	—	—
Cardueae	26	11	—	—	—	—	9	—	—	—	—
(Arctoteae)	—	—	—	—	—	—	—	—	—	—	—
(Mutisieae)	—	—	—	—	—	—	—	—	—	—	—
Cichorieae	9	—	—	1	—	—	3	—	—	—	—

<sup>x</sup> GE = Germacranolides, EL = Elemanolides, SA = Santanolides, ER = Eremophilanolides, BA = Bakkenolides, GU = Guaianolides, XA = Xanthanolides, AM = Ambrosanolides (= Pseudoguaianolides), PS = Psilostachyanolides, and VE = Vermeeranolides

Table 2. Distribution of polyacetylenes in Compositae (Gottlieb, 1982)

<i>Tribe</i>	$C_8$	$C_9$	$C_{10}$	$C_{11}$	$C_{12}$	$C_{13}$	$C_{14}$	$C_{15}$	$C_{16}$	$C_{17}$	$C_{18}$	<i>Total</i>
	-	-	9	-	-	-	-	-	-	1	1	11
Cynareae	2	-	14	6	18	74	45	23	19	27	9	237
Senecioneae	1	-	5	7	11	8	7	1	1	13	4	58
Muticeae	-	-	-	-	5	6	1	-	-	1	-	13
Astereae	1	2	56	3	4	1	3	1	5	22	-	98
Anthemideae	-	6	37	18	35	50	57	7	6	22	6	244
Helianthaceae	-	3	4	4	28	118	19	9	8	25	-	218
Calenduleae	-	1	-	-	-	4	1	-	-	-	-	6
Eupatoricae	-	1	1	-	-	7	1	-	-	-	-	10
Inuleae	-	-	4	-	7	27	6	-	-	-	-	44
Vernonicae	-	-	-	-	-	7	-	-	-	-	-	7
Arctoteae	-	-	-	7	24	25	20	-	-	-	-	76

## EFFICIENT SEED IRRADIATION AND MUTANT SELECTION WITH EMPHASIS ON PEANUT AND SORGHUM\*

Joventino D. Soriano\*\*

### Introduction

It has been some 50 years or so since man realized the useful possibilities of what was then known as unseen rays or ionizing radiation for producing genetic variability in living things. The eventual control of radioactivity by physicists at the University of Chicago in the early 1940's led, after World War II, to the easy availability of irradiation facilities for the artificial induction of mutations. Some mutation laboratories, however, took some time to acquire the new irradiation facilities. Up to 1962, for instance, the conventional x-ray machine was still the only radiation source at the mutations laboratory of Professors Robert A. Nilan and Calvin F. Konzak at Washington State University, two prominent names in plant mutagenesis at the time.

With many seed irradiation facilities available in many countries by the mid-1960's, the induction of mutation attracted the interest of many biologists, agronomists and plant breeders whose pioneering work founded the field of mutation breeding. It did not take long, however, before mutation workers realized the need for basic studies on the nature of radiation damage or injury on cells and tissues to obtain repeatable and predictable results. Problems related to what was then known as "oxygen effect", "after-effect" or "extra-radiation effect" were extensively investigated during the past two decades. As a result, it is now possible to treat seeds with relatively high and effective radiation doses with minimum and acceptable degrees of biological damage.

Seed irradiation and mutant selection afford workers in general and biologists in particular a better understanding and appreciation of the intricate and fascinating phenomena related to genetic variability — nature's way of providing the innumerable, interesting and useful kinds of flora and fauna that inhabit our planet. Induced or artificial mutation is highly relevant to both developed and developing societies in relation to the plant breeder's dilemma of needing entirely new germplasm for improving the economic potentials of food and industrial crops

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\*Paper presented at the *State-of-the-Art* Seminar Series of the Biological Sciences Division, National Academy of Science and Technology, held at the NAST Conference Room on December 9, 1985.

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and helping alleviate the enormous problems brought about mainly by the ever-increasing human population.

There are today more than 500 crop varieties that have originated through mutation breeding (Donnini *et al.*, 1984) within a time span of only about 30 or so years. Of this number, about nine out of every ten of such cultivars were induced through seed irradiation and the rest by bud, tuber and pollen irradiation. The popularity of seed irradiation is invariably due to the relative ease of seed treatment, the capacity of seeds to withstand high and effective radiation doses, and the tolerance of seeds to a wide range of temperature, chemical and other treatments without incurring heavy reductions in seed viability.

### Efficient Seed Irradiation

Extra-radiation damage has been considered one of the most important causes of set backs in the mutation breeding work on seeds. It has been estimated that under ordinary conditions, the magnitude of such damage may even surpass that caused by radiation alone and may result in total inviability of seeds. Particularly for the sparsely ionizing radiations, like x-rays and gamma radiation, the magnitude of extra-radiation effects could be twice or thrice the injury due to radiation treatment itself. No such damage has been reported with the use of densely ionizing radiations like fast neutrons, except those obtained under strictly rigid conditions of certain experimental tests.

#### *Pre- and post-irradiation treatments*

Appropriate protocols have been adopted to bring about maximum efficiency in seed-irradiation treatments through modification of biological damage. These are the maintenance of a seed moisture content of approximately 12-14 percent prior to and during radiation treatment, post-irradiation soaking of the treated seeds in water at a warm temperature of about 30°C for about two hours and enhancing embryo growth soon after rehydration (Nilan *et al.*, 1961). Without the plant radiobiologists' efforts to unravel the nature of radiation damage, inducing mutation through seed irradiation would have continued to be the "hit and miss" type of work characteristic of the 1950's and 1960's.

Our data (Table 1) shows that at high doses, radiation damage in  $M_1$  plants as indicated by reductions in seedling height and seedset, was markedly higher in plants that did not receive the pre- and post-irradiation treatments than in those given such treatments. Moreover, the mutation frequency was markedly higher in plants given the pre- and post-irradiation treatments than in those that did not receive such treatments with the same radiation dose.

It is now widely known that the indirect effect of radiation is hydrolysis of water through the action of primary radicals, resulting in the formation of peroxides and other harmful compounds. In moist seeds, the enhanced movement

Table 1.  $M_1$  seedling height and seedset and  $M_2$  mutation frequency in sorghum with pre- and post irradiation treatments

Gamma radiation dose	Without pre and post irradiation treatment			With pre- and post-irradiation treatment		
	Mean $M_1$ seedling ht. (% Control)	Mean $M_1$ seedset (% Control)	Mutants per 1000 seedlings	Mean $M_1$ seedling ht. (% Control)	Mean $M_1$ seedset (% Control)	Mutation per 1000 seedlings
0 (Control)	100.00	100.00	0	100.00	100.00	0
15 Krads	100.00	91.47	5.62	100.00	100.00	7.01
30 Krads	87.46	75.72	10.37	92.64	94.78	18.16
45 Krads	61.45	66.24	17.63	79.27	82.04	39.74
60 Krads	26.78	39.05	9.46	58.16	65.59	29.32

of such radicals toward their reunion minimizes their reaction with oxygen to form the harmful molecules. Thus, reductions in seedling growth, plant growth and fertility invariably result from damage of cells in the apical meristems which are transmitted from one cell generation to the next in the form of chromosomal breakage and other forms of genetic injury induced by exposure to ionizing radiation. Reduction of the frequency of mutation observed in the  $M_2$  generation most likely results from the formation of inviable  $M_1$  (first mutagen generation) gametes and  $M_1$  seed embryos that presumably bear the mutations.

#### *Intrasomatic competition*

In addition to the foregoing means of modifying the effects of radiation on seeds to reduce biological damage, efficiency of mutation could also be enhanced through proper management of the distribution of mutated cells or sectors in the  $M_1$  meristems so that the mutations in such cells and sectors will be included in the ensuing seed. Not a few mutation workers now realize that there is more than mere exposure of seeds to radiation and waiting for useful mutant plant types to appear in the population.

Actually, from the time the plumule of the treated seed begins to break its dormancy to the time of fertilization and seed formation, "a thousand and one" cellular processes contributing to the inclusion of a mutation in the  $M_2$  seed embryo take place. It has been estimated, for example, that more than a million cell divisions take place in a growing plant from germination to maturity. While most of these biological processes are presently beyond man's control, at least one inter-cellular mechanism, i.e., the competition between normal and mutated cells in the meristem appears to be a promising venue for increasing the chances of an induced mutation participating in seed formation.

The data in Table 2 show the important influence of  $M_1$  planting distance on mutation frequency in the  $M_2$  generation. Given the same radiation dose,

Table 2.  $M_2$  mutation frequency in peanut as influenced by  $M_1$  planting distance

<i>Gamma radiation dose</i>	<i>Wide planting, 50 x 50 cm</i>		<i>Close planting, 10 x 10 cm</i>	
	<i>Total <math>M_2</math> seedlings</i>	<i>Mutants per 1000 seedlings</i>	<i>Total <math>M_2</math> seedlings</i>	<i>Mutants per 1000 seedlings</i>
0 (Control)	0	—	0	—
15 Krads	1	.52	5	3.71
30 Krads	4	2.32	12	6.57
45 Krads	9	4.79	23	23.90
60 Krads	3	2.89	14	7.53

close planting yielded mutation frequencies that were 2-7 times more than wide planting. The  $M_1$  plants in the latter were about 5 times farther apart than those in the former. At a planting distance of about 50 cm between rows and in the row, from 6 to 9 basal branches developed per plant while at a distance of only about 10 cm between rows and in the row, the  $M_1$  plants had only 1-3 branches each. The mutation frequencies were obtained from about one-half of the branches per plant selected at random.

Intrasomatic competition (Gaul, 1964) is based on the idea that the apical meristems of plants originating from irradiated seeds are composed of both normal and mutated cell initials. Sectoral chimeras, however, are not stable and are likely to be overgrown by normal tissues during the period of growth. As mutated cells are mostly injured or damaged genetically, they are distinctly at a disadvantage compared with normal cells which invariably undergo the processes of division, enlargement and differentiation at a normal rate.

Through natural selection, mutated cells will likely become eliminated as often evidenced by the eventual loss or disappearance of chimeral leaf sectors and their replacement by normal tissues. By planting  $M_1$  plants close together, overcrowding and partial shading occur. There is also possible stiff competition for water and soil nutrients among tightly interwoven root systems of neighboring plants that are grown very close together. As these conditions are not conducive to the fast growth of normal tissues, the mutated sectors have more or less an equal chance of surviving and eventually becoming transmitted to the next generation.

Another aspect of intrasomatic competition refers to the influence of planting distance on tillering rate. In tillering grassoids like rice, wheat and barley, close planting discourages tillering while wide planting promotes tiller development. With many tillers in the plant, the chance of a tiller bearing a mutated sector is much less than when only a few tillers are formed.

#### *Distribution of mutations*

Attempts to apply the principle of intrasomatic competition in non-tillering grassoids and aerial-branching dicotyledons were, however, unsuccessful. Dissi-

milar mutation frequencies in various parts of an  $M_1$  plant of these types indicate that a related phenomenon, herein called mutation distribution, may serve to explain these differences.

It may be important to know how mutant cells or sectors are distributed in the plant body during the period of growth. If this information is known, it might be possible and advisable to manage the distribution of mutated cells or sectors in the plant for the sole purpose of obtaining the highest mutation frequency in the progeny.

Apparently, different plant groups have varying types of mutation distribution schemes based on the mutation rates from pods or fruits harvested from different parts of the plant. The type of distribution of mutations is probably governed by branching habit, such as alternate, opposite or dichotomous branching. By pruning the lower or upper branches or cutting back the plant's main axis, it might be possible to concentrate the induced mutations in a small part of the plant's body and minimize their loss during the growth period.

The type of distribution of induced mutations, genic or chromosomal, which probably varies in different species groups, is presently being investigated in the Botany Experimental Garden at the University of the Philippines campus in Quezon City using three leguminous species with different branching habits, namely, peanut (base branching), soybean (alternate branching) and sweet peas (no branching). Legumes grow in clayey soil like that in the Diliman area. Seeds from different parts of the plant will likely give different levels of mutation frequency. If the portion of the  $M_1$  plant bearing the highest frequency of mutations is known, it will be quite possible to manage or direct the occurrence of high rates of genetic changes after mutagenic treatment.

### **Efficient Mutant Selection**

The search or detection of mutants in a population with a mutagen-treated background constitutes a major part of the protocol in mutation breeding work. Induced mutations are by nature more or less alien or new to the existing environment and, therefore, need human intervention for their survival. While most of the listed mutations have been selected visually, a good number of them required some sophisticated means of detection and quantification like those involving high protein and oil contents, protein quality, baking quality, fragrance quality and others. There seems to be a mutation for almost every human desire for a good life which is a possible indication of the innumerable number of germplasm variations existing in plants.

The seemingly unlimited genetic properties coded in existing crop species and their wild relatives present exciting possibilities for the human race given the time, ingenuity and patience to unlock such codes through mutation breeding. Certain genetic phenomena, however, appear to control the expression of some

types of mutations, such as delayed segregation and the occurrence of several degrees of disease resistance which could initially mask the detection of the mutant for high resistance to the disease. As the method of screening disease resistance employed in our work on peanut and sorghum are of the classical and traditional type, only the phenomenon of delayed segregation will be discussed here.

### *Delayed segregation*

Some mutant characters while not appearing in the  $M_1$  generation show up in later generations, while others appear in the  $M_1$  population but fail to show up in succeeding generations. As shown in Table 3, of the five mutant characters induced in peanut through gamma seed irradiation, dwarfness, dark-green, short internodes and viny appeared in the  $M_1$  generation but failed to show up in the second or third generation. The appearance of white testa in later generations but not in the  $M_1$  generation suggests the need for continuous or recurrent selection in order to identify the useful mutations.

In this connection, the failure of a mutant character to appear in the next generation has been commonly referred to as mutant reversion, an assertion allegedly first found in mutant bacteria and extrapolated to higher organisms without any valid evidence that did or does occur.

Of more than 500 registered mutations in higher plants, no mutant reversion has been reported. An "off-type" character should first be crossed with the wild-type and give evidence of segregation or recombination before it can be recorded as a mutation. With the 390 dwarf plants and 106 dark-green plants found in the  $M_1$  generation, a layman will likely jump to the absurd conclusion that those are cases of mutant reversion. As it will turn out, not all "off-type" or new characters found in the  $M_1$  and other mutagen-treated generations are valid mutations until they pass the crucial test of segregation with their wild-type alleles. Otherwise, it will be premature to consider such characters as mutations. A similar observation has been reported in peas (Vassileva *et al.*, 1983) where early flowering and big seed obtained in the first treated generation were later found to be genetically controlled.

The delay in the segregation of some induced characters may be due to the highly heterozygous and heterogenous nature of the plant with a mutagen-treated background. Although most induced mutations are genetically monogenic, many have been found to be controlled by duplicate genes while others are multi-allelic, polygenic and epistatic in nature (Cooper and Gregory, 1980; Donnini *et al.*, 1984; Palmer *et al.*, 1978; Soriano, 1984). An induced mutation of the monogenic type in the heterozygous state requires two or more generations to segregate, depending on the genetic composition of the generative cells. The expression of mutations requiring additive action of many genes, as well as those governed by duplicate or epistatic factors, will likely require more than one generation to appear in the population.

Table 3. Frequency of five "off-type"  $M_1$  characters in peanut after gamma seed irradiation in different generations

<i>Plant character</i>	<i>M<sub>1</sub> generation</i>		<i>M<sub>2</sub> generation</i>		<i>M<sub>3</sub> generation</i>		<i>M<sub>4</sub> generation</i>	
	<i>No. of "off-type" plants</i>	<i>%</i>	<i>No. of segregating lines</i>	<i>%</i>	<i>No. of segregating lines</i>	<i>%</i>	<i>No. of segregating lines</i>	<i>%</i>
Dwarfness	396	2.28	6	.005	2	.002	0	—
Darkgreen	115	0.66	9	.007	0	—	0	—
Short internodes	22	.001	0	—	8	.008	0	—
Viny	79	.005	0	—	0	—	2	.003
White testa	0	0	9	.007	1	.0009	5	.007

### Future Research Directions

The major problems encountered at present in mutation breeding, such as lethality following treatment of seeds with high but effective doses of radiation and the low transmission of induced mutations to the progeny, may be minimized by the employment of *in vitro* biotechniques, a growing field of biological investigation. Seeds treated with radiation are sprouted for a few hours or days and small cell groups or tissues from the root or shoot meristem are grown aseptically in appropriate culture medium. Explants are gradually acclimatized for growth under greenhouse conditions.

Somatic cell culture offers the distinct advantage of generating whole plants from single cells, cell groups, protoplasts and protoplast hybrids produced through parasexual hybridization. The isolation of protoplasts that could undergo proliferation by cell division originally reported in soybean, carrot, maize and barley is now a widely accepted technique in many laboratories. Interspecific hybridization of protoplasts has been successfully done in crosses between oats and maize, soybean, broad bean and many other combinations.

Mutation workers could further explore the possibility of growing immature  $M_1$  seed embryos on sterile medium (Bajay *et al.*, 1970; Bouhermont, 1961) and rescue them from the lethality that usually results from treatment with high but mutagenically-effective doses of radiation. The culture of such embryos has become a practical method after the discovery about a decade ago that the quantity of endosperm material in the immature seed does not influence the success of the culture. Related to this method is the possible production of haploids from  $M_1$  anther and pollen cultures, a technique already successfully demonstrated in 23 species of higher plants including such familiar crop species as rice, sorghum, oats, barley and tomato.

The direct transfer of foreign genetic materials such as mutated chromosomes, DNA and genes into eukaryotic cells, i.e., wall-less protoplasts, may be employed by future mutation breeders to insure the expression of mutant characters in irradiated lines which would otherwise be lethal. This possibility has been indicated by the successful uptake of radioactive-labelled genetic materials by protoplasts of higher plants like *Petunia hybrida* and *Ammi Visnaga* (Smith, 1974). The transfer of genes from mutated microorganisms to protoplasts and cells of higher plants is now a legitimate possibility. In fact, the *in vitro* culture of mutant chromosomes, DNA and genes themselves may someday become the one single omnibus method of widening genetic variability in economic plants which today is achieved through conventional breeding supplemented by mutation breeding, with the latter being done mainly through seed irradiation and mutant selection.

### Summary and Conclusion

Recent developments in seed irradiation research consist of (1) the employment of pre- and post-irradiation treatments to modify radiation damage and there-

by increase the efficiency of inducing mutations, (2) proper management of the distribution of mutations to insure the transmission of mutational changes to the progeny, and (3) continuous or recurrent selection to obtain even the genetic changes that segregate in advanced generations. Use of *in vitro* biotechniques in mutation breeding programs will likely become the ultimate possibility for inducing mutant germplasm useful in improving economic plants for the benefit of man.

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## CURRENT EFFORTS IN PLANT ECO-PHYSIOLOGY\*

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### ABSTRACT

The effort in eco-physiology in the past decade (1975-1984) focused on two main areas: weeds and their control, and plant nutrition and fertilizer application. A few other areas (such as water relations, flowering, growth regulation) were touched on, but the effort was still in the nature of ground-breaking.

The biology and ecology of various weed species, farm management practices as means to control weeds, herbicide application and crop-weed competition were phases of the weed problem which were given attention. Although herbicides produced spectacular results, their procurement presents a grave concern because the country is short in foreign exchange.

The standard studies in plant nutrition dealt with the essential elements owing to the widely accepted view that the soil is a source of nutrients for plant growth. However, attention was recently given to the possibility that the soil could also be a source of environmental stress. Some of the problems were the presence (in excess) of an essential element like iron, harmful levels of aluminum and other non-essential elements, and salinity. The fertilizers used by farmers were largely nitrogenous; less attention was given to phosphorus and potassium fertilizers; and minor elements were rarely, if at all, incorporated in fertilizers. Experiments during the decade were apparently designed to relate with this farming practice. Some studies demonstrated the beneficial effects on coconut of chloride-containing fertilizers. The fertilizer needs of coconut was assessed by leaf analysis.

Water relations in plants is the basis of practices in irrigation and drainage. Cognizant of the problems posed by the semi-arid climate in the western side of the country, researchers studied the response of plants to drought. Apparently, the ultimate objective was either to identify some drought tolerant varieties (ecotypes) or to list down some characteristics associated with drought tolerance.

Confirming the results of studies abroad, local researchers demonstrated the importance of daylength in inducing plants to shift to the reproductive phase. In addition, a few of the growth regulators were shown to be effective in inducing flowering.

Growth regulators are attractive materials for study because they are very effective at dilute concentration. They bring about increased growth in elongation, rooting of cuttings and many other functions. Their effects may be exerted through a change in enzymatic activity.

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\*Paper presented at the *State-of-the-Art* Seminar Series of the Biological Sciences Division, National Academy of Science and Technology, held at the NAST Conference Room on March 21, 1985.

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### Scope of the Review

We propose to cover the area of plant biology which borders between ecology and physiology. Emphasis will be laid on those studies concerned with the reaction of field plants, or studies designed to improve current field practices. This area in plant biology is sometimes called crop physiology, and at other times, agro-ecology or agricultural ecology. We will skirt those laboratory physiological studies on the internal changes and processes of the plant, such as the pathways of photosynthesis or of intermediary metabolism.

Most of the local studies in plant science are in crop physiology for three main reasons. First of all, agriculturists tend to pursue science not only "to advance the frontiers of knowledge" but also to improve agricultural practice. Secondly, local technology and the manufacturing industry cannot yet sustain studies requiring intricate laboratory material and facilities. Such studies would have to be sustained through a pipeline (as it were) from abroad, and could entail much expenditure of foreign exchange. Thirdly, experiments in crop physiology do not require of a fledgling researcher much preparation in technique and skill. And yet, a researcher in eco-physiology needs unusual talent to comprehend the inter-play of multi-variate, uncontrolled factors.

The review will cover the studies published in the current decade (1975-1984). Prominence will be given to studies on weeds and their control, and on inorganic plant nutrition including use of fertilizers, because they constitute the main concern of the current research effort. In addition, a section will deal with the less-sustained, disparate studies in the other areas of eco-physiology in order to indicate beach-heads which could be exploited in the future.

### Weeds and their Control

By definition, any plant which is not wanted in a place is a weed. However, in practice, any plant which is useful, or which gives an economic return is wanted; hence, is not considered a weed. From a layman's point of view, a weed is a hardy, nuisance plant which competes with a crop. For example, Alabang X is considered a weed among field and orchard crops, although it is a good pasture grass. Manuel (42) enumerated some of the characteristics of weeds in rice fields which make them harmful and persistent. She mentioned that annual weeds start to flower at 5 weeks after the rice is transplanted. Soon after this, they produce a multitude of seeds, insuring the build up of weeds in the next cropping season. Although largely propagated by seeds, weeds also multiply through rhizomes, tubers, bulbs, corms, stolons or off-shoots.

Weeds either directly or indirectly cause expenditure in cultivation, cover cropping, herbicides, irrigation, drainage and other practices. Heretofore, weeds have not puzzled farmers because being always visible they are easily sized up. The

damage they inflict is considered a result of the farmer's neglect. In contrast, insect pests and diseases are alarming when they strike because they seem to come from nowhere.

In previous years, interest was focused on the flooded field because there was an effort to inject science in lowland rice farming. But since 1975, workers have been shifting interest to the drained field owing to the realization that the weed problem in the drained field is greater in number and kind than in the flooded field. Moreover, the information gathered on the former would have a wider application because majority of the farms are non-irrigated – not to mention the fact that crops other than rice are being planted in the drained field. And since the drained field is more amenable to mechanization, the farmer could work a bigger area of land should a scheme of effective weed control be designed for this kind of environment.

The broad scope of the studies may be categorized into the biology and ecology of weeds, farm management practices designed to control weeds, herbicides and their application and competition between crop and weed. An overall assessment of the effort is appended to serve as basis for further cogitation.

### *Biology and ecology of weeds*

The biology of a few important weed species was studied to discover some vulnerable aspect in their lives – the Achilles' heel, as it were – which would make them easy to control. Mercado, Manuel and Pamplona (48, 49, 50, 59, 60, 61, 62) studied the life cycle of "aguingay", (*Rottboellia exaltata* L.f.) an important weed in the fields of sugar-cane, corn and legumes. Samples of seeds were obtained from these fields and from a "ruderal" area, in order to study their seed characteristics, and growth habit of the plant. These workers distinguished five eco-types based on the shape, size and color of seeds; on the period of dormancy; on the hairiness of the collar of seedlings; and on the number of tillers, age at flowering, number of spikes, height and dry weight of the mature plant. All the ecotypes flowered in short days; their critical day-length was 13 hours. Water extracts from various parts of the plant were shown to inhibit the germination of *Ipomoea triloba* seeds, but less so, of *Phaseolus radiatus*. The inhibitory substance was apparently heat-stable.

Another troublesome weed species is *Paspalum distichum* L. From a rhizome, it could grow fast and attain maximum dry-matter production in 40-80 days after planting (43, 44). The plant can also reproduce by stolon. Being largely mesophytic, it does not grow well in flooded fields. By planting the crop densely or by planting a dense-growing rice variety (such as IR-42), the farmer can minimize damage from this weed.

Among the non-grass species, *Portulaca oleracea*, *Sphenoclea zeylanica*, *Pistia stratiotes* and *Eichhornia crassipes* attracted incidental attention. Ociones (55) attributed the dormancy in *Portulaca* seeds to the impermeable seedcoat and

immature embryo. Germination was promoted by scarification of the seedcoat, or by exposure of the seeds to sunlight while in the seedbed. On the other hand, germination of *Sphenocloa* seeds was enhanced either by heating to 40°C for 30 minutes, by treatment with gibberellic acid (1.0 ppm), or by treatment with potassium nitrate (10.0 ppm). Growth of the resulting seedling was most rapid at 2-8 weeks after transplanting (34).

*Pistia* and *Eichhornia* are floating aquatics which clog Laguna de Bay and the Pasig River at certain times of the year. In their study, Bua-ngam and Mercado (18) found that seeds of *Pistia* could germinate under water. After 20 days of submergence, the young plant broke through the seedcoat, its collar leaf expanded, and it subsequently floated. In two months of growth, 180 offshoots were produced from a single plant. They observed that the spadix was initiated 63 days after transplanting. Spadices were located at the center of the leaf whorl, numbering 3 to 8 per plant. Flowers were borne singly at leaf axils, subtended by a spathe-like bract (52). The flower was zygomorphic, perfect, with monocarpellate ovary, containing 4-15 ovules. The stamen had 4-8 anthers. Seeds were produced in 135 days after transplanting, at the rate of 4-12 seeds per spadix. The seed was oblong, with wrinkled seedcoat; it had an air-chamber to make it buoyant.

Talatala and Soerjani (71) reported that subjecting the flowers of *Eichhornia* to wind current for 30-60 minutes brought about pollination and production of seeds; but not at shorter duration. Depth of water did not affect seed production. Calcium seemed to be essential to seed development.

In the ecological study, the presumption was that volunteer weeds in a fallow field were most adapted to that particular niche. Hence, one may gain an idea of the kind and magnitude of the weed problem by making an inventory of weeds in a field.

Sajise *et al.* (66) decided to study the ecology of a natural grassland because grasses present the most serious weed problem in cultivated fields. On the basis of counts made in their quadrat, they tentatively distinguished four types of communities; namely, *Themeda triandra* type, *Imperata cylindrica*, *Capillipedium parviflora*, and *Chrysopogon aciculatus*. Each of these types was considered as a stage of a so-called ruderal vegetation — i.e., a vegetation which arose as a result of the frequent recurrence of an agent of destruction, such as fire. There were indications that one type could shift to another type, depending upon changes in the environment. For example, application of high rates of nitrogenous fertilizers favored a shift from the *Imperata cylindrica* type to the *Themeda triandra* type. Overgrazing tended to favor the *Chrysopogon* type.

The weed problem in the rice fields of Nueva Ecija was assessed through an opinion survey of farmers' estimate of serious weed problems. The results are shown in Table 1. The highest number of farmers considered *Echinochloa crusgalli* as serious in the irrigated field, while *Ischaemum rugosum* was considered serious in the rain-fed fields. The other serious weeds were *Paspalum distichum* and *Monochoria vaginalis* in irrigated fields, while *Fimbristylis miliacea*, *Echinochloa crusgalli*

and *Monochoria vaginalis* were serious in the rain-fed fields. The serious weeds in other provinces are shown in Table 2. *Fimbristylis miliacea* and *Cynodon dactylon* were cited as common in Pangasinan, Cagayan Valley and Iloilo.

Table 1. Problem weeds in rice fields of Nueva Ecija (Cabanatuan City and Guimba) as identified by farmers in 1979 (23)

Weed species	Number of farmers reporting	
	Irrigated	Rain-fed
<i>Echinochloa crus-galli</i>	61	19
<i>Paspalum distichum</i>	18	4
<i>Monochoria vaginalis</i>	12	14
<i>Sphenoclea zeylanica</i>	2	1
<i>Fimbristylis miliacea</i>	4	27
<i>Ceratophyllum demersum</i>	1	—
<i>Ischaemum rugosum</i>	2	30
<i>Eichhornia crassipes</i>	1	—
<i>Cyperus rotundus</i>	—	2
<i>Ipomoea aquatica</i>	1	5
<i>Echinochloa colona</i>	—	2
<i>Panicum repens</i>	—	5
<i>Brachiaria mutica</i>	—	2
<i>Leersia hexandra</i>	—	1
<i>Cyperus</i> sp.	—	1

Table 2. Problem weeds in rice fields reported by farmers in 1979 (30)

Weed species	Manaoag, Pangasinan	Cagayan Valley	Iloilo
<i>Monochoria vaginalis</i>	✓	✓	—
<i>Cyperus difformis</i>	✓	✓	—
<i>Fimbristylis miliacea</i>	✓	✓	✓
<i>Commelina diffusa</i>	✓	—	—
<i>Echinochloa colona</i>	✓	✓	—
<i>Cynodon dactylon</i>	✓	✓	✓
<i>Ipomoea aquatica</i>	—	✓	—
<i>Echinochloa stagnina</i>	—	✓	—
<i>Ischaemum rugosum</i>	—	—	✓

The kind and magnitude of the weed problem is apparently affected by the ability of the soil to impound water. The higher the ponding potential, the less is the total weed population (Table 3). In well-drained fields *Paspalum dilatatum*, *Eleusine indica* and *Dactyloctenium aegyptium* were dominant; in fields with very low ponding potential, *Echinochloa colona* and *Calopogonium mucunoides* dominated. The field with low ponding potential and that with high ponding potential were dominated by *Leptochloa chinensis*.

In the vegetable patches of Baguio, Balaki (11) observed sub-temperate weed species such as *Galinsoga parviflora*, *Poa annua* and *Spergula arvensis*. Moreover, some of the cosmopolitan species such as *Ageratum conyzoides* and *Portulaca oleracea* seemed to have distinct lowland (warm) and highland (cool) ecotypes.

With few exceptions, the species found in the carrot fields of Baguio were also found in those of Mountain Province and Nueva Vizcaya.

Table 3. Weight of weeds in grams per square meter from fields with different ponding potential. Dry-seeded rice in Los Baños, 1977. (3, 4, 5, 6, 7)

Weed species	Ponding potential of the land			
	Well-drained	Very low	Low	High
<i>Paspalum dilatatum</i>	136.4	—	—	—
<i>Echinochloa colona</i>	5.6	232.8	—	8.8
<i>Leptochloa chinensis</i>	25.2	—	172.0	127.2
<i>Eleusine indica</i>	90.8	9.2	—	—
<i>Calopogonium mucunoides</i>	12.8	69.2	—	—
<i>Cyperus iria</i>	—	—	6.0	—
<i>Monochoria vaginalis</i>	—	—	—	27.6
<i>Paspalum fasciculatum</i>	—	10.0	—	—
<i>Portulaca oleracea</i>	—	8.4	—	—
<i>Dactyloctenium aegyptium</i>	48.4	—	—	—
	385.0	353.0	180.0	170.0

### Farm management practices

Standard farm practices are mainly intended to make certain aspects of the environment favorable to the crop. In addition, they incidentally put down the weeds. A few of these practices are plowing, harrowing, and puddling during land preparation, hilling up and off-barring, dense planting, multiple cropping, cover cropping, crop rotation, mulching, irrigation and drainage, fertilizer application, and handweeding, mowing or rolling.

Land preparation gives the crop a head-start by killing the weeds or setting back their growth. During cultivation of the crop (hilling up and off-barring) the weeds are either uprooted or set back when their root system get disturbed. By planting densely, the farmer leaves very little space for the weeds to grow. In multiple cropping, the space left vacant by the main crop is taken over by the inter-crop; hence, no space is left for the weed to grow. Crop rotation or sequential cropping may be arranged so that one crop would benefit the succeeding crop in terms of reduced weed population. The mulch may exclude sunlight on the soil surface, thus preventing germination of weed seeds. When the mulch begins to decay, the carbon dioxide tension on the soil surface will rise and asphyxiate the germinating weed seeds. Flooding may adversely affect some weeds, while drying may likewise affect other species. Fertilizers may preferentially increase the growth of the crop, enabling it to smother the weeds. The weed is uprooted and disposed of by hand weeding; its growth is set back by cutting, or by rolling. It is hard to allocate credit to each of these operations because in most studies they are combined. The task is made harder by the practice of reckoning the success of a weeding operation in terms of increased crop yield instead of decreased weed population. It could be argued that the test of the pudding is in the eating, but the eating of the pudding could be affected by the appetite of the eater and other factors. Similarly, the yield might be conditioned by the potential of the crop and by other constraints in the environment.

Olofintoye and Mabbayad (36) compared the effects of various tillage practices on weeds and on yield of upland rice. They found that conventional tillage was more effective than the other tillage regimes. The dominant weeds in this treatment were *Leptochloa chinensis* and *Commelina bengalensis*, occupying 41 percent of the plot area. Conventional tillage gave the highest yield (Table 4). The least effective was zero tillage. The dominant weeds were *L. chinensis*, *C. bengalensis* and *Cleome rutidosperma*. Minimum tillage favored *C. rutidosperma*, while delayed seedbed favored *L. chinensis*. The general impression drawn from this experiment was that herbicides cannot replace thorough land preparation.

In the lowland rice field, it was shown once again that harrowing immediately before transplanting was more effective in putting down the weeds than harrowing two or three days before transplanting. The problem did not differ in severity among plots harrowed once, twice or thrice (31, 32).

In upland cotton, inter-row cultivation by hoeing at 2-4 weeks after emergence, coupled with handweeding, provided a satisfactory means of weed control. However, using boll weight as criterion of result, inter-row cultivation in combination with the use of herbicides gave better results than inter-row cultivation alone (65).

In a study where twelve weed control approaches were compared in a field of corn, it was found that (a) removing the weed flush of growth with a directed spray of paraquat at 25 days after seeding (DAS) followed by hilling up at 32 DAS gave yields comparable with that of handweeding; (b) in terms of net return per

Table 4. Effect of tillage regime on percentage of plot area covered by weeds and on computed yield (56)

Criteria of results	Tillage regime			
	Conventional tillage	Zero tillage	Minimum tillage	Delayed seedbed
1. Percent of plot area covered by:				
a. <i>Leptochloa chinensis</i>	22	40	24	65
b. <i>Eleusine indica</i>	10	10	21	20
c. <i>Commelina bengalensis</i>	19	33	22	19
d. <i>Cleome rutidosperma</i>	3	36	72	4
e. <i>Mimosa pudica</i>	0	3	11	1
2. Yield of rice in tons/ha	3.7	2.2	2.3	2.6

Conventional tillage – one plowing, one harrowing, two rotavation, and handweeding at 20-40 days after seeding

Zero tillage – no tillage, herbicide was applied

Minimum tillage – one rotavation, herbicide was applied

Delayed seedbed – one plowing, one rotavation, herbicide was applied

peso invested, the treatments which compared favorably with handweeding were: (b.1) animal-powered harrowing followed by paraquat at 25 DAS and hilling up at 32 DAS; (b.2) animal-powered harrowing, followed by off-barring at 14 DAS and hilling up at 32 DAS; and (b.3) paraquat followed by off-barring at 24 DAS and hilling up at 32 DAS (25).

Pablico and Moody (57, 58) grew six crops in two years in a drained field as part of a study on crop rotation (cropping pattern). Their results are summarized in Table 5. The total weight of weeds, in grams per square meter, did not consistently change with the cropping pattern. Although *Amaranthus spinosus* was dominant in the first crop, it decreased in importance in the succeeding crops so that at the end it constituted only a fraction of one percent in many cropping patterns. *Rottboelia exaltata* was not conspicuous in the first crop, but starting with the second crop of the rice-sorghum sequence it became a major constituent of the weed population (36.5, 83.4, 57.3 and 73.4 percent). *Ipomoea triloba*, *Echinochloa colona* and *Cyperus rotundus* became noticeable in various seasons but they did not form a trend.

In a series of studies on weeds in cropping systems affected by landscape position (ponding potential) and weeding regimes, Ahmed and Moody (8) found that the effect of landscape positions were: (a) Grasses predominated in all fields regardless of ponding potential. They constituted 58.7 to 89.5 percent of the weed

Table 5. Effect of crop rotation (cropping pattern) and cropping season on total weeds and on *A. spinosus*

Season/ Criteria	Cropping Pattern						
	1	2	3	4	5	6	7
1. 1977 wet season							
a. maize	rice	rice	sorghum	maize	mungbean	mungbean	
b. 378.8	699.2	549.2	523.6	448.0	967.6	704.8	
c. 76.7	83.8	75.7	81.6	83.8	91.2	90.4	
2. 1977 wet season							
a. maize	maize	sorghum	sorghum	rice	rice	mungbean	
b. 484.4	210.8	270.8	370.8	537.6	396.4	252.8	
c. 64.7	4.1	0.5	73.4	88.2	87.7	90.4	
3. 1978 wet season							
a. maize	rice	rice	sorghum	maize	mungbean	mungbean	
b. 456.0	521.6	766.8	563.6	850.0	338.0	534.0	
c. 65.0	41.6	11.9	94.5	76.6	75.1	70.0	
4. 1978 dry season							
a. maize	maize	sorghum	sorghum	rice	rice	mungbean	
b. 134.4	168.0	287.6	107.2	135.2	81.6	240.8	
c. 11.1	42.3	22.1	18.3	31.1	23.0	5.7	
5. 1978-79 dry season							
a. maize			sorghum	mungbean	mungbean	mungbean	
b. 141.6			101.6	267.2	214.0	113.2	
c. 52.3			26.0	7.6	22.6	29.3	

Table 5 continued

Seasonal/ Criteria	Cropping Pattern						
	1	2	3	4	5	6	7
6. 1979 wet season							
a. maize	maize	maize	maize	maize	maize	maize	maize
b.	345.2	261.6	842.8	514.8	1,169.2	424.4	448.4
c.	6.3	0.5	0.4	2.7	0.4	0.4	0.8

- a. Crop
- b. Total weight of weeds in grams per square meter at five weeks after planting. Data were obtained on unweeded plots (weeds not removed).
- c. *Amaranthus spinosus* in percent of total weed weight.

flora, on wet weight basis. (b) The weight of the broad-leaf weeds decreased as the ponding potential increased. (c) There were less weed species in fields previously puddled than in fields prepared dry. (d) Grain yield increased in control plots with increase in ponding potential. (3, 4, 5, 6).

In another experiment with dry-seeded wetland rice, Ahmed and Moody (2) found that weeding at 3 to 4 weeks after seedling emergence resulted in yields which were comparable with those from plots kept weed-free. Balyan, Bahn and Singh (13) independently arrived at the same observation. In their study, the drilled upland rice was handweeded 30 days after sowing. It seemed that the proper timing of the weeding operation was more important than the frequency of weeding.

In the direct-seeded rice of the rain-fed field of Central Luzon, the natural density of *Echinochloa colona* was 280 plants per square meter or 2.8 million per hectare. Maximum tillering occurred, during the first 40 days after seeding, and each plant produced 42,000 seeds. The seeds were shed in trickles and were in various states of dormancy. Because they germinated in flushes over a wide range of time, they were very hard to control (51).

*Paspalum distichum*, a weed in lowland rice fields, is rather troublesome because it reproduces by seed, stolon and rhizomes. From a rhizome cutting, Manuel and Mercado (44) observed that the average shoot production was one per day; shoot elongation, 1.0-1.5 cm per day; and leaf production, 40 per day. The peak of dry matter production occurred at 40-80 days after planting.

The indications from two cropping seasons were that in the rain-fed rice field of Laguna (International Rice Research Institute) *Rottboellia exaltata* was dominant; in Pangasinan and Iloilo, *Echinochloa colona* was dominant. By hand-weeding the first crop, the researchers brought about a change in weed dominance in the following crop. *Echinochloa colona* became dominant in Laguna, and *Cyperus iria*, in Iloilo; there was no change in Pangasinan (30).

In Muñoz, Nueva Ecija, and in San Idefonso, Bulacan, the rice-tomato, or the rice-mungbean cropping pattern did not have an effect on the kind and amount of weeds. The rice variety IR-38 was better than IR-36 in keeping down the weeds.

It was speculated that by breaking the dormancy of seeds, weeds can be made to germinate simultaneously; hence, the seedlings can be killed all at once. Occiones (55) found that scarification, heating and light treatment were a few methods of breaking the dormancy of Portulaca; gibberellic acid and potassium nitrate treatments had no effect. Laganao (34) found that heating the seeds of *Sphenoclea zeylanica* at 40°C for 30 minutes enhanced their germination; gibberellic acid and potassium nitrate treatment did likewise.

There was only one study in Los Baños on mulching (28). In a tomato field heavily infested with *Rottboellia exaltata*, rice straw, rice hull, sawdust and sugar-cane bagasse were compared. None of the mulches was able to suppress the weed. When laid out thickly, the materials reduced the population of *Cyperus rotundus* and of *Ipomoea triloba*.

Various soil moisture levels and depth of flooding (up to 5 cm) were compared in their capacity to suppress the weeds in rice fields. *Echinochloa colona* persisted in all water treatments; *Digitaria setigera* and *Cyperus iria* succumbed to early flooding at a depth of 5 cm and *Monochoria vaginalis* and *Sphenoclea zeylanica* succumbed to late flooding (24).

Phosphatic fertilizers applied to mungbean fields did not increase the weeds in terms of weight per unit area; nitrogenous fertilizers increased the weight of weeds. To minimize weeds and maximize yield, the optimum distance of mungbean should be 50 cm between rows.

Although expensive, handweeding seemed essential even in plots treated with herbicides (1). The critical period for the control of weeds seemed to be the first four weeks after planting. Weeds which sprout after the fourth week become shaded out and inflict minimum damage.

### *Herbicides and their application*

The two-fold objective in applying a herbicide to a field is to prevent the germination of weed seeds and/or to kill the weeds which have already germinated. Farmers often overlook the likelihood that the herbicide can adversely affect the crop and that the environment can render the herbicide ineffectual. These angles have been explored by different workers.

Balyan *et al.* (13) found that Pendimethalin applied at 1.5 kg/ha as pre-emergence spray, followed by handweeding 30 days after sowing, was most effective in controlling weeds in drilled upland rice. However, the highest rice yield was obtained when the pre-emergence treatment consisted of Pendimethalin (1.5 kg/ha) and oxidiazon (1.0 kg/ha) followed by handweeding.

Pendimethalin had a higher residual effect in Lipa clay loam than in Guadalupe clay, or in Taal fine sandy loam. The rate of loss was slower at low soil moisture levels. It was high during the first 20-50 days after application; it tapered off thereafter. Pendimethalin is used against *Rottoboelia exaltata* (16, 17).

Lopez (38) studied the toxicity of Atrazine, 2, 4-D isopropyl ester (IPE), and Linuron to corn, *Eleusine indica*, *Ipomoea triloba*, *Cyperus rotundus* and *Amaranthus spinosus*. Atrazine and 2, 4-D IPE were toxic to all species at pH 6.2-7.2; however, after 2 and 4 weeks of incubation, they were no longer toxic. Linuron at 0.9 to 1.33 ppm was toxic to cucumber but less so, when incubated for 2 to 4 weeks.

The moisture level of the soil did not affect the action on the cotton crop of Diuron, Fluometuron and Prometryne when applied as pre-emergence spray (64). However, they adversely affected the crop when incorporated mechanically with the soil. The percentage of germination, weight and height of the seedlings were reduced.

Pre-emergence treatment with Prometryne, Linuron and Fluometuron at 4 kg/ha, provided satisfactory control of *Eleusine indica* in cotton fields (26).

However, the boll production in plots which received the herbicide was lower than that in plots kept weed-free by handweeding.

In carrot fields, the most effective dose was 1.0 kg/ha of Linuron applied as pre-emergence spray, and repeated at 30 days after planting (11).

Diphenamid (4-6 kg/ha) controlled the weeds in peanut without injury to the crop (72). Watering the pots at 3 to 6 days' interval favored the crop and aggravated the damage of the herbicide on the weed.

Oxidiazon applied to the soil at 1.0 kg/ha or higher, was detrimental to sorghum, even if this was planted 30 days after the treatment (69). The toxic effect of the herbicide was much reduced on the crop planted at 60 days after treatment, but growth was still less than the untreated. Some of the factors which were presumed to have contributed to the diminution of the toxic effect were high temperature, higher solar radiation and the adsorptive capacity of the soil. In field trials, Oxidiazon had little or no adverse effect on rice and a sedge (*Scirpus maritimus*) but was quite effective against grasses and broadleaves. (69).

It is curious that some herbicides antagonize each other. Treatment of rice seeds (IR-36 and RD-19) with Naphthalic anhydride provided the best protection of the seedlings against the toxic effects of Thiobencarb. The latter inhibited root growth and produced, kinked row of cells in the mesophyll, which should have been linear in the developing leaf. Other promising herbicide protectants are Naphthamide and GA<sub>3</sub> in low concentrations. They stimulated shoot elongation (70).

Naphthalic anhydride (NA) increased root length of rice seedlings, which would have been retarded by treatment with Butachlor, Molinate, Pendimethalin or Thiobencarb (9). A similar neutralizing effect of NA was observed in corn seedlings treated with Butylate, EPTC and Vernolate. However, it failed to protect soybean from Pendimethalin injury. Applied alone, NA was phytotoxic to sorghum and soybean.

The rice seedlings of variety Nam Sagui are relatively tolerant, while those of C-168 are susceptible to Butachlor. In the tests of Noriel (54), both were severely affected when 50 ppm of the herbicide was applied as pre-emergence and as post-emergence spray. The herbicide was more toxic at high than at low moisture level in the soil.

Trifluralin seemed to be more toxic to the four ecotypes of *Rottboelia exaltata* than Pendimethalin (12). The effective rates for both were 1.0 to 1.5 kg (active ingredients)/ha. When used in a soybean field, Trifluralin at 1 kg/ha did not inhibit nodulation of the crop when incorporated with the soil 10 days before sowing; but was detrimental when applied 2 days before sowing. In either case, *R. exaltata* was effectively controlled.

In the corn field, Pendimethalin was able to control 60 to 90 percent of the weed (*R. exaltata*) when the crop was at the two- and three-leaf stage. At an earlier stage, especially at the coleoptile stage, corn was adversely affected. Older than the three-leaf stage, both the crop and the weed were not adversely affected by Pendimethalin.

Napropamide was most effective against *Cyperus rotundus* in soils of medium texture (33, 37). When the tubers of the weed were planted deep and the soil was treated with the herbicide, the shoots which emerged were fewer and shorter than those which emerged in the untreated control. There were indications that  $\alpha$ -amylase and germination of the tuber were inhibited when Napropamide was at 10 ppm or higher.

*C. rotundus* was likewise controlled by pre-emergence application of Naptalan (45). Applied post-emergence in a series of concentrations (up to 8 kg/ha), it progressively increased the number of aerial shoots, tubers and rhizomes. However the shoots were small and vulnerable to subsequent control treatments.

In a soybean field, *Ipomoea triloba* was selectively controlled by Bentazon applied sequentially at 2.0, 2.0 and 3.0 kg/ha. If applied only once, the herbicide was ineffective (10).

Among the aquatic plants, *Monochoria vaginalis* and *Pistia stratiotes* were treated with herbicide. Granular 2, 4-D was effective in controlling the former when applied at the 4-leaf stage or older. Butachlor was effective when applied at pre-emergence, while Thiobencarb was not effective at all (35). On the other hand, *P. stratiotes* was controlled satisfactorily by either Diquat (1 kg/ha), Glyphosate (3 kg/ha), or Piperophos dimethametryn (0.6/0.15 kg/ha) (19).

At sub-herbicidal concentration, some benefits may be derived from these growth regulators. At 0.05 to 0.15 mg/kg of soil, Simetryne and Benzomarc tended to increase the dry-matter production of rice; nitrate reductase and the nitrate content of the leaf-blade were likewise increased. The optimum effect was noted 2 weeks after treatment (27). A similar increase in nitrate nitrogen was induced in papaya by the application of Diuron and Ametryne. At 4 kg/ha, they did not adversely affect the growth of papaya, but tended to reduce the chlorophyll content of the leaves (46).

Pendimethalin when incorporated with the soil at the rate of 1.0 kg/ha resulted in high accumulation of phosphorus in soybean seedlings; at 3.0 kg/ha, lowest accumulation (67).

Incidental benefit from herbicides was indicated by the reduction of damage from Rhizoctonia damping off by Trifluralin and Prometryne on cowpea (14, 53); from Sclerotium damping off by Napropamide and Butalin on tomato (15); and from rust by Trifluralin and Bentazon on soybeans (39).

### *Competition between crop and weed*

Ecologists visualize three general kinds of interaction between two species (say, the crop and the weed) which grow together; namely, positive, neutral or negative. However, farmers are generally interested in the negative effects of the weeds on the crop; seldom in the negative effects of the crop on the weeds; and not at all, in the positive effect of the weed on the crop.

When soybean was planted in pots with 1 to 4 seedlings of *Ipomoea triloba*, it was not adversely affected by the weed in terms of plant height or number of days to first flowering; however, there were reductions in number of leaves, number of branches, flowers and seeds, the seed weight, and the dry weight of the whole plant. Among the varieties tested, BPI-L-114 gave the highest yield when kept weed-free; with 4 weeds per pot, variety Clark 63 gave the highest yield, followed in the descending order by UPLB-Sy-2, TK-5 and BPI-L-114 (40).

Corn planted in pots with 1 to 6 seedlings of *I. triloba* showed various capacity to compete. Variety Phil DMR Comp-2 suffered least reduction in yield, followed by UPCA-3, UPCA-5 and UPCA-1 (20, 21) in increasing order of yield reduction.

Cotton was planted in pots with 5/10/20 plants of some weed species. Compared with the weed-free control, the yield reduction caused by the weed was 74 percent for *Echinochloa colona*; 45 percent for *Cyperus rotundus*; and 35 percent for *Trianthema portulacastrum* (29).

#### *An overview of the studies on weeds*

It is heartening to note that more and more science is being harnessed towards the control of weeds. Heretofore, the only recourse open to farmers was the back-breaking task of uprooting the weeds by hand or with simple implements. Because of the inefficiency of handweeding, weeds have been a major limitation to the area which a farmer could till; moreover, they impelled a primitive farmer to abandon his kaingin every three or four years. At present, more effective and indirect means of weed control are being tried. The studies on the comparative biology and ecology of the weeds and the crops could yield very interesting results, not to mention their usefulness in field practice. If it can be shown, for example, that a top dressing of nitrogenous fertilizer can bring about a marked difference between the growth curve of a legume crop and that of a grass weed in favor of the former, then an early fertilizer top-dressing can give the crop some competitive advantage. In one other aspect of competition, the possibilities of using plants which exude allelopathic substances may be explored. There are other aspects of competition which may be utilized to reduce the cost of weed control.

Regarding soil management, it is common knowledge that a field which has been cropped continuously for some time has a less serious weed problem than one which is more recently reclaimed from the wild (i.e., grassland or forest). While this knowledge has impelled farmers in Europe and America to culture their crops in greenhouses and sheltered gardens, we have not taken to this practice. Perhaps it would be relevant to study the operation of a sheltered garden, not only from the standpoint of weed control but also for its profitability.

Much of the study in this review concerned the use of herbicides, either alone or in combination with certain standard farm practices. Some of the results were spectacular and provided strong argument in favor of using herbicides. One

likely realization however is that the country has to pay for herbicides in foreign exchange. Rather than allow this consideration to dampen our interest in herbicides, we should encourage local chemists to initiate moves to produce at least a few of them.

### **Plant Nutrition and Use of Fertilizers**

Farmers have had a long and sustained interest in the use of fertilizers because these increase spectacularly the growth and yield of crops. As agricultural practices became more and more scientific, the initially empirical use of fertilizers was gradually superseded by systematic practices based on scientific knowledge. This knowledge was obtained from studies in plant nutrition.

#### *Local studies in plant nutrition*

There have been occasional studies in plant nutrition since the 1920's when Dr. R.B. Espino introduced here the technique of growing plants in solution culture. Most of the studies were designed to catalogue the symptoms of deficiency in a particular element. To induce deficiency, the element was withheld from the culture solution. The information was considered useful in diagnosing the fertilizer needs of a crop from its appearance in a field.

In the past decade, interest in plant nutrition has veered towards abnormalities in the crop brought about by the presence of deleterious substances in the soil. In other words, interest in the soil as a source of material for plant growth has shifted to interest in it as a stress to plant growth. Thus, on hypothesizing that the cadang-cadang (blight) disease of coconuts was caused by some detrimental factor in the soil, Velasco and his colleagues analyzed the soil of affected groves. After many false leads, they were able to report that the disease seemed to be associated with low levels of the rare earths (lanthanides) in the soil (123). Further observations on the rare earths (125, 126) tended to confirm this initial finding. However, they felt that the complex situation in Bicol where abnormal symptoms differed from one species to another could have been caused by the superimposition of the effects of some other element on those of the rare earths. At one time they reported the presence of thallium (124), but this was soon belied by alternative confirmatory tests. Further explorations are being made and lately there are indications that chromium may be present.

The seasonal yellowing of rice in some areas attracted the attention of Solivas and Ponnanmperuma (117). They traced the cause to a toxic excess of soluble iron, abetted by the presence of acid sulfates. The standard remedy is to lime the soil, but the operation could be expensive. An alternative approach which they tried, was to search for a tolerant variety. In their mass screening of varieties, a few varieties proved tolerant to high levels of iron.

Like iron, high levels of aluminum can be troublesome in certain fields. Zaini and Mercado (129) approached the problem by growing plants in culture solution. A tolerant variety of rice (IAC-3) and a susceptible variety (IR-45) were grown in solutions with different ratios of aluminum to calcium. It was found that at high aluminum and low calcium, the tolerant variety had higher concentration of aluminum in the root and lower concentration in the shoot than the susceptible variety. This was interpreted to mean that in this variety there was more immobilization of aluminum in the root and less translocation to the shoot than in the latter.

Still another source of environmental stress is the presence of much salt (NaCl) in the soil solution. In rice fields inundated by sea water, rice seedlings turn yellow, are unthrifty and hardly bear grains. The problem is especially acute during the dry season. Rodrin and Mercado (111) took three varieties known to differ in their ability to tolerate salt, and grew them in culture solution. They found that the  $\alpha$ -amylase activity could be an indicator of salt tolerance. IR-8, a salt-tolerant variety, was least inhibited in its  $\alpha$ -amylase activity by the salinized medium; PB76TB188, a moderately tolerant variety, was moderately affected; and C4-63, a susceptible variety, most adversely affected. Pretreatment of the seeds with calcium chloride before germinating them in the salinized medium seemed to have improved the  $\alpha$ -amylase activity in C4-63 and in PB76TB188 but not in IR-8. Salination at 0.1, 0.2, and 0.3 percent (weight/volume basis) caused immediate reduction of ribonucleic acid (RNA) in the three varieties. Deoxyribonucleic acid (DNA) increased with salination up to the second week; then decreased thereafter. At high levels of salinity, the shoot was more depressed than the root.

Deficiency in an essential element, like presence of an unwanted element, can be a form of environmental stress. Plants may modify their structure, or their composition in response to the stress. Thus, when three rice varieties (IR-8, C4-53G and BPI-76) were grown in RS-17 culture solution in which the levels of the elements varied, root growth was highest in P-deficient plants, followed in the descending order by K-deficient, complete, N-, Mg-, and Ca-deficient plants. Chlorophyll was highest in plants grown in complete culture solution, followed in the descending order by P-, K-, Ca-, Mg- and N-deficient plants. Highest nitrate content was found in the leaves of K-deficient plants (105).

The drying of leaf tips and yellowing of plants around Tadolak Lake, Los Baños, was suspected to be due to the high concentration of elemental sulfur in the spring water (90), coupled with high temperature. The different species varied in susceptibility to these factors.

#### *Field tests with nitrogenous fertilizers*

In most efforts to improve soil fertility, nitrogenous fertilizers are used cause (a) most soils are deficient in nitrogen, (b) the response of plants to added

nitrogen is spectacular, and (c) nitrogen is easily lost by evaporation as gaseous nitrogen or as ammonia.

A study by Alimagno and Yoshida (75) to increase the nitrogen content of the soil by inoculation with nitrogen-fixing blue-green algae showed that although blue-green algae increased total nitrogen in the soil, they did not affect favorably the growth and yield of rice. It was likely that the nitrogen was still in an unavailable form, or that the algae competed with rice for some of the soil nutrients.

In upland rice culture, 120 kg N/ha split into three doses gave the highest yield, provided that the weeds were controlled. There was indication that deep placement of nitrogen, as mudball, led to more efficient utilization, but it may not be practical. On the other hand, Malabuyoc *et al.* (97) found that 40 to 80 kg N/ha gave significant increases in grain yield. Their study was conducted for four years in three scattered sites. It showed that good crop is obtained if upland rice is planted within one month from the onset of the rainy season.

In lowland rice culture, nitrogenous fertilizers have low efficiency. To improve the efficiency, two slow-release fertilizers (silica-polymer-coated, and sulfur-coated) were compared with the usual split application of urea (81). In the 1980 dry season, the grain yields from the slow-release nitrogen fertilizer and from the deeply-placed urea did not differ significantly. They out-yielded the usual split method of applying 2/3 of the fertilizer broadcast before transplanting and 1/3 top-dressing at panicle initiation. Proper timing of a 50 kg N/ha application could match the 112 kg N/ha applied in the usual split method.

Wheat variety Neveh Yaar and Acc. No. 4073 gave the highest yield when given a rate of 160 kg N/ha, but the optimum rate was 80 kg N/ha. The best time of planting was January 23, which allowed the crop to enjoy the cooler temperature coupled with low relative humidity (120).

The performance of three mungbean cultivars was variable (in a trial comparing inoculation with *Rhizobium* versus fertilizer application at 20 kg K/ha. Some cultivars responded favorably to inoculation while others did not. It was shown that fertilizer application at planting was not necessary (101). Mungbean fertilized with P and K (0-60-60 kg/ha) and given full illumination gave the highest number of pods per plant; at 75 percent illumination, it gave the highest number of nodules; and at 50 percent illumination, the highest mean grain yield (119).

On the other hand, soybean seemed to perform well when given a complete fertilizer (20-60-10 kg NPK/ha) coupled with inoculation. Its yield was reduced, despite the fertilizer, if not inoculated (114).

In a fertilizer study, chicken manure was compared with commercial fertilizer (14-14-14 kg NPK/ha) applied to Irish potato in Baguio. The beneficial effect of chicken manure was most marked in subsoil or soil which tended to be dry (106). Commercial fertilizers increased the yields in 6 sites but not in 7 sites. Another study showed that potassium was critical in some Baguio soils for potato. In sandy clay loam, a potash content of 0.73 me/100 g soil was considered sufficient; below this, potash was needed (115).

Nitrogenous fertilizer increased the alpha-cellulose and hemi-cellulose of one abaca variety (Pacol X CES 3) more than those of Tinawagang Puti. With phosphorus fertilizer (50 kg P/ha), both varieties contained high holocellulose and alpha-cellulose (78). Kenaf (variety NSDB 63-1) was planted in Maahas clay and fertilized with 75-50-50 kg NPK/ha. The best fiber yield was obtained at a population density of 300,000 plants/ha (79). With ramie, the best population density was 50,000 plants/ha at 90-60-60 kg NPK/ha planted in Maahas clay loam (80).

An extensive sugarcane fertilizer trial involving 8 varieties and 15 locations was conducted by Philsugin. All the varieties showed definite response to nitrogen fertilization, but increases in yield differed among varieties. The main effect of nitrogen was in increased tonnage. The response to phosphorus and potassium was not distinct, or there was no response at all (121). Population density and water regime were studied in relation to nitrogen fertilization (112, 85). It was found that nitrogen did not have an effect on root growth, but spacing between furrows (lower than 0.75 meters) reduced root growth. In Angeles fine sand, irrigation every 15 days gave the best results. More frequent irrigation resulted in lower tonnage and sugar rendement. The best fertilizer formula was 300-100-300 kg NPK/ha. Increasing the nitrogen to 400 resulted in decreased yield, when potash was not concomitantly increased (85). One report stated that foliar spray of urea could replace as much as 75 percent of soil-applied nitrogen. Starting at 2 months after planting, four sprayings were made at fortnightly intervals. As much as 20 percent urea solution could be sprayed without harmful effect (118). However, these field results of Songplung and Rosario were not confirmed by Cardenas and Rosario (82) under greenhouse conditions. They found that only the most dilute solution (1 percent) caused no leaf injury; 3 and 6 percent solutions caused leaf injuries. On the basis of non-response to urea-spray in terms of plant height and leaf-area per plant, they concluded that foliar application of urea was ineffective on sugarcane.

Instead of vegetative growth response, mango responds to nitrogenous foliar spray by producing flowers. Potassium nitrate at 10-40 g/li induced flowering in the variety Carabao when sprayed on shoots, 4.5 to 8.5 months old. The older the shoot, the more responsive it is to the spray; at 8.5 months (dark-green and crispy leaves), 10 g/li solution was sufficient to induce flowering. At 40 g/li, there was a reduction in percent flowering, panicle length, number of flowers and sex ratio. Heavy rains and typhoons delayed flowering (77).

#### *Effects of chloride and other fertilizers on coconut*

Since 1971, there had been reports abroad that the chloride ion was beneficial to coconuts. Among local workers, attention to this ion started to build up sometime in 1975. Prudente and Mendoza (107) noted marked response of coconut to potassium chloride in their NPK fertilizer trial in inland field in Davao. After minimizing the contribution of potassium in the yield increases, they came to the

conclusion that the increases were due to the chloride ion of the salt. They went further in showing big positive partial correlation between leaf content of chloride ion and copra production. As a follow-up study, table salt was added in increments of 10 grams per plant in Tugbok clay contained in poly-ethylene bags. There was a proportionate increase in the girth of the seedling with the increment of salt applied. Analysis for chloride of the third leaf from the spear indicated that the critical level of chloride was 0.7 to 0.8 percent. Chloride seemed to render the seedlings resistant to leaf spot disease (*Pestalozzia palmarum*) (95). This observation on resistance build-up by addition of the chloride ion (this time from KCl) was confirmed by Abad *et al.* (73), who studied six-year-old palms and fully-bearing palms. The unfertilized plants (control) and those receiving nitrogen alone and nitrogen plus phosphorus had significantly higher number of spots than the chloride-treated plants. Other studies (98, 96, 104, 76) confirmed the beneficial effects of added chloride fertilizer. The critical chloride level in the leaves of fully-bearing coconut was placed at 0.5-0.55 percent.

Santiago (116) grew coconut seedlings for five months in three types of soil fertilized at four levels of nutrient concentration. The clay loam initially contained 1487 ppm N, 16 ppm P, 220 ppm K, 98 ppm Cl, 6.04 me Mg/100 g soil, and 15 me Ca/100 g soil. The corresponding concentrations for silty clay loam were 1636, 9, 667, 98, 4.75 and 14; and for sand, 1736, 24, 136, 98, 2.89 and 3.85. Fertilizers were added to bring up the various nutrients at the same level in three soil types. It was found that the three soil types and the four fertility levels did not affect the vigor of the seedlings, nor the level of leaf N, P, K, Cl and Mg, but significantly influenced the leaf-Ca.

Kamala Devi and Velayutham (89) tried to find how soon coconut leaves reflected the effects of fertilizer application. With nitrogen fertilizers, the 14th leaf attained a maximum of 1.98 %N on the fifth day, while the unopened leaf attained 2.19 %N in the same period. The increase in N was 19-32% in the 14th leaf and 21-36% in the latter. There was no effect on the outer leaf. With phosphorus, the maximum (attained on the fifth day) was 0.17 %P in the 14th leaf, 0.146 %P in the unopened, and 0.139 %P in the outer leaf. The maximum for potassium (attained on the second day after application of the fertilizer) was 2.32 %K in the 14th leaf and 3.15%K in the unopened. There was little or no change in the leaf-content of the other elements incident to fertilizer application.

Local studies on leaf analysis (92, 94) tended to follow the method and concepts developed in the Ivory Coast. By analyzing the 14th leaf for the essential nutrients, Magat reported that the trees in Laguna, Quezon, Leyte, Davao and Zamboanga which yielded 65 or more nuts per tree per year contained nutrients which equalled or exceeded the suggested critical levels of 1.8-2.0 %N, 0.12 %P, 0.8-1.0 %K, 0.5 %Ca, 0.3 %Mg, 0.5 %Cl, and 0.2 %S. In the fertilizer trial in Davao and Misamis Oriental low-yielding groves (less than 50 nuts/tree/year) there was positive response in terms of nut and copra production starting two years from initial fertilization. This response was closely related to improvement in leaf nitro-

gen and chloride levels, but not in P, K, Ca and Mg levels. It was interpreted that the soil was deficient in the former two nutrients and sufficient in the latter four nutrients. Magat proposed to scale down the critical levels for Ca, Mg, and S to 0.3, 0.2 and 0.15%, respectively.

Wahid *et al.* (128), reviewing the results of phosphorus fertilizer trials in a majority of coconut-growing countries during the last 50 years, concluded that phosphorus application had no significant positive effect on either nut yield or copra. They suggested that future attention be directed to finding (1) the effects of discontinuing phosphorus application on bearing trees, (2) the comparative effectiveness of various forms of phosphate fertilizers, (3) the amount of extractable P with various extractants and (4) the relation among time and method of application and form of P on one hand and soil pH, method of planting and rooting pattern on the other.

Magat (93), in an extensive review of fertilizer studies on coconut presented three approaches to determining the fertilizer needs; i.e., soil analysis, nutrient deficiency symptoms, and leaf analysis. Reporting on fertilizer practices in various countries, he emphasized the exciting results with the chloride ion in the Philippines. He noted that in the Ivory Coast potash gave big increases in yield; despite this observation, the workers recommended a balanced NKMg fertilizer. It was only in Sri Lanka where response to phosphate was reported. In India, a balanced NPK mixture seemed best for the sandy soil in Travancore-Cochin.

### *The micro-nutrients in field studies*

Most soils have enough reserves of micro-nutrients; hence, farmers pay little attention to the latter in fertilizer practice. In the few instances where a micro-nutrient is deficient, they fail to recognize the problem and usually apply more NPK hoping to improve the growth and yield of the crop – only to aggravate the situation further.

The discovery that some rice areas in the Philippines are deficient in zinc came about incidentally as a result of shot-gun fertilizer application. Subsequently, more systematic studies were made. Navarro and Kirschy (102) found that at one site in Pangasinan, rice failed to produce when zinc was not added. The yield did not differ when given either 8 or 16 kg Zn/ha. They seemed to note a Zn-P interaction, where P tended to depress the effects of Zn. Raising seedlings in Zn-sufficient areas or dipping the roots in zinc solution before planting in the Zn-deficient fields seemed to have alleviated the situation but did not solve the problem completely (109). In some poorly drained soils of Laguna, Rizal and Leyte, organic matter was high and available P was low. Rice responded well to application of NPK fertilizer; however, even greater yields were elicited by supplementing the NPK with copper, molybdenum and zinc (108).

Preliminary, unpublished results of the writers pointed to a deficiency of boron in eastern Cavite. Sitao, squash, okra, eggplant and pepper seem to be very

sensitive to the deficiency, especially during the dry season. On the other hand, gabi, camote, papaya and banana seem to be tolerant.

Miniano and de Guzman (100) demonstrated the beneficial effects of the chloride ion on non-makapuno embryos grown *in vitro*. However, more definitive information is needed to establish the essentiality of chloride to coconut.

In passing, it is worth mentioning that incorporation of charcoal in the growth medium improved the growth of makapuno embryo *in vitro*. Instead of a chemical effect, charcoal must have affected some physical process.

### Glints from the Rest of the Spectrum

Other than weed control and fertilizer application, a broad spectrum of studies in eco-physiology awaits more than passing notice. However, for some reason, the various aspects have not been given the attention they deserve. For example, water relations in soils and plants are basic to the practice of irrigation and drainage; the shift from the vegetative to the reproductive stage is important in the culture of crops which produce economic returns in terms of grains and fruits; and growth regulators (accelerators and inhibitors) are the new wave in scientific agriculture. The writers will attempt to present the modest effort in these areas in the following discussion.

#### *Water relations*

The western side of the Philippines is classified as a semi-arid region. During six months of the year (June to December), excessive rains present a problem of water disposal, while during the rest of the year, there is drought. Rice grows best during the wet season, hence our vast rice areas. However, the fields lie fallow during the dry season because we have not found a good crop which could withstand the drought, nor have we designed a system of farming which would effectively utilize the limited water supply.

Rao and Datta (171) planted the rice variety Jaya in a sandy loam and provided it with various water levels. They obtained best yields at 5 cm and 0.5 cm levels. At 15-20 cm water level, there were less productive tillers, less grain per panicle and lighter grains. In their survey of rice varieties for drought resistance, Parao *et al.* (168) suggested that this character is related to the ability of the variety to develop a deep, well proliferated root system. Deep rooting could be approximately expressed by root-to-shoot ratio (dR/S) — i.e., mg root measured 30 cm below the soil surface per  $\gamma$  shoot. The ratio is related to tiller number but not to plant height.

To study the relationship between the nitrogen nutritional level of rice seedlings and their response to water stress, Baldia (134) raised two lots of plants in 10

ppm N and in 40 ppm N and subjected them to decreased osmotic potential. The latter was effected by adding PEG 600 (poly-ethylene glycol) to the solution, which was separated from the roots by a semi-permeable membrane. With the lowering of the osmotic potential, there was a decrease in shoot growth. Osmotic stress enhanced root growth — more in low N than in high N level. In the control solution (-0.6 bar), the dawn leaf-water potential (full turgor) of plants in 10 ppm N was higher by 1.3 bars than in the 40 ppm N.

Rice, corn and soybean were subjected to water stress by growing them in pots and withholding water at 19 days after planting the rice, and 12 and 15 days after planting corn and soybean, respectively. Water stress significantly decreased leaf elongation, leaf water potential, transpiration and nutrient uptake. The crops were more tolerant to water stress during the rainy season than during the dry season. Of the three species, rice was least tolerant (183). Nine varieties of corn were subjected to moisture stress at the vegetative and at the reproductive stage. Based on the so-called cluster analysis, it was shown that varieties responded differently when water was withheld from them for six days. But in general, they were more resistant to moisture stress during the vegetative than during the reproductive stage. Moisture stress tremendously lowered the yield when imposed at, or a few days before, silk emergence (143).

The water status of plants was studied by the relative water-content technique, where leaf discs were floated in water. It was shown that the flotation temperature of the water-affected water uptake, increased with increase in temperature confirming previous finding. This could be explained by oversaturation and growth of leaf tissue at high temperature. In turn, oversaturation is associated with initial rate of uptake and water deficit, while growth may involve cell-wall relaxation (164).

Using the pressure-chamber technique, Samson and Pacardo (177) compared water relations in six tree species. The trees differed with respect to osmotic potential, water potential, intracellular water volume, bound water percentage, and modulus of elasticity of the cell-wall. According to their ability to withstand drought, *Leucaena leucocephala*, was followed by *Samanea saman*, *Wrightia lanitibia*, *Albizia procera*, *Gliricidia sepium*, and *Gmelina arborea*, in the descending order.

Transpiration and leaf resistance of some crops were studied by subjecting them to a wind velocity of 150 cm/sec. (in a wind tunnel) and light intensity of 5500 lux. Based on loss in weight, cotton had the highest rate of transpiration, followed by corn, tobacco, soybean and sorghum in the descending order. Leaf resistance, measured by the porometer method, was inversely related to transpiration (159).

Soil moisture, solar radiation, rainfall and air temperature were monitored for 9 months to detect differences among some upland ecosystems and relate them with the morpho-physiological characters of the resident species. It was found that the slope (30, 50 and 70 degrees) affected the soil moisture in various ecosystems, except the grassland. There was highly significant negative correla-

tion between soil moisture content and soil temperature in the grassland, new and old kaingin ecosystems, but not in the plantation and the secondary forest ecosystems. The relationship between soil moisture content and relative leaf water content was positive in *Gliricidia sepium*. Relative leaf-water content and leaf-moisture content were positively correlated in *Carica papaya*, *Imperata cylindrica* and *Gliricidia sepium*. (185).

In culture solution, tomato exhibited a reduction in relative leaf-water content, leaf-moisture content and leaf-water potential at a water stress of  $-1.25$  bars, while mungbean suffered a reduction in those parameters at  $-2.5$  bars. In other words, mungbean is more resistant to drought than tomato. The stomata in the abaxial surface of both species closed at the same moisture stress; however, in the adaxial leaf surface, the stomata of tomato remained open at  $-1.25$  bars, while those in mungbean were already closed. Hence, the latter could conserve more moisture under increased water stress and be more drought-resistant. Under field condition, mungbean exhibited no reduction in economic yield when grown under low soil water condition. It is most sensitive to water stress only up to 45 days after germination (178).

In *Phaseolus vulgaris* L. cv "White Baguio", water stress reduced stem elongation, leaf growth, seed and fruit yield, it delayed flower formation. However, it increased chlorophyll content (180).

Seedlings subjected to temporary drought developed some change in their functions so that they could withstand drought in the later stages of life. When tomato and pechay were hardened, Rasco (172) found that growth rate, shoot-root ratio and rates of transpiration differed from the unhardened ones. There was more free proline in hardened plants; this is an indirect measure of drought resistance.

Nine hours after harvest, leafy vegetables were allowed to wilt at  $22^{\circ}\text{C}$  and 60% relative humidity, so that changes could be monitored. Wilting increased the total sugar and free proline level; but decreased percent protein and starch. Protein was significantly decreased in Chinese cabbage (140). When Irish potato (*Solanum tuberosum* L.) was subjected to moisture stress at 30, 45 and 60 days after planting, the tubers were poorer in quality and less marketable. When stress was applied only once, the total yield was not markedly reduced (139).

### *Flowering and fruiting as affected by daylength and other factors.*

Knowledge of the factors which govern flowering and fruiting, and the processes involved in the shift from the vegetative to the reproductive stage can have much economic value. In the flower business, a farmer's ability to force plants to flower to avail of a good market can give him much competitive advantage. Exotic fruits can be raised in a locality if plants can be induced to fruit outside their natural environment. Native plants can be made to produce more,

without increasing the total dry-matter produced, by simply shifting the grain-to-straw ratio or fruit-to-shoot ratio.

One of the most widely used methods of inducing flowering in plants is to give them their photo-inductive daylength. In rice, most lowland varieties are sensitive to short days. In their survey of photoperiod sensitivity among upland varieties, Alluri and Vergara (132) found that of the 30 varieties, 17 were insensitive to photoperiod, 5 weakly sensitive and only 8 strongly sensitive. Two varieties which are photoperiod sensitive (BE-3 and BKN6819-36-3-1) were compared with two non-sensitive ones (IR-36 and IR-747B2-6-2) as regards yield response to planting date and seedling age at planting. IR-36 outyielded all the other varieties in all treatment combinations. Both non-sensitive varieties showed an abrupt yield decline when 40-day old seedlings were used. Seedling age at planting had no effect on the performance of sensitive varieties.

To determine the effect of low light intensity on ratooning, rice plants were subjected to 0 shading (full sunlight), 49% and 66% shading from flowering until maturity. Ratoon yields from the unshaded plants were 34% higher than those from plants with 66% shading (145).

The test with three varieties of winged beans showed that at 9 hours of light, there was 100% flowering in all varieties. Grown in 12.75 hours (neutral day), UP Selection 22 required 5 short days to effect flower-bud initiation; Batangas medium, 7 short days; and Pangasinan long, 11 short days (155). Given 10-20 short days, Pangasinan long effected 100% flowering initiation. One short day followed by one long day resulted in fewer and smaller flowers (154). In the variety UPS 22, abscisic acid hastened flowering, while gibberellic acid (GA-3) delayed it (153).

Cotton seems to be a short-day plant (157). The 20-day old plants exposed to 10 hours of light flowered earlier by 2.6 days than those in 12 hours, and 6.8 days earlier than those in 15 hours. In the 10-hour photoperiod, plants were tallest, had the most branches, and produced the highest seedcotton.

Gibberellic acid (GA) sprayed on cabbage at the hook stage (approximately 60 days after sowing) induced elongation of the stalk in both varieties (Leo 80 and KK). However, only KK flowered (60-70%) when sprayed four times with 500 to 1,000 ppm (174). Analyzed by the dwarf rice seedling technique, endogenous GA was highest in cabbage at 50-90 days after sowing. GA in the latter age was correlated with flowering. Application of exogenous gibberellic acid brought an increase in the endogenous level of GA and in auxin, but not in cytokinin (148).

Growth regulators may affect the sex ratio of flowers (179) or the fruit quality (175).

### *Growth regulators and enzymes which affect development*

Nitrate reductase activity in the leaves of corn was shown to be significantly correlated with grain yield, dry matter production and harvest index (150).

When pechay, mustard, radish, lettuce and cabbage were fertilized with different levels of nitrogen fertilizers, nitrate accumulated significantly in the edible parts. Nitrate reductase was also increased. It was suggested that the ratio between the induced activity of nitrate reductase and the normal activity could be an index of nitrogen utilization (165).

Tests with ripeners and deterrents on sugarcane showed that invertase could be greatly inhibited by some of these agents. They could prolong the storage time of the cane before milling (152). Among the chemical ripeners tested on sugarcane, Bualta and MBR 12825-45 were found effective (187).

Since makapuno coconut is apparently a result of excessive proliferation of cells, del Rosario (173) looked into the status of endogenous growth regulators in its liquid endosperm. She found that in both the carrot phloem tissue assay and the soybean callus bioassay, makapuno had more growth-promoting activity than the liquid endosperm of normal coconut. Moreover, cytokinin-like substances were demonstrated to be present in makapuno liquid endosperm. In hormone analysis, paper chromatography and filtration chromatography on Sephadex IH-20 were employed. When makapuno embryo was cultured *in vitro*, de Guzman *et al.* (149) observed that seedling growth was inhibited or made abnormal by the presence of 2, 4-D in the medium. The root was more adversely affected than the shoot. However, 2, 4-D could induce extensive proliferation of cells and tissues leading to disorganization and callus formation. Working further with explants *in vitro*, de Guzman *et al.* (146) supplemented Murashige and Skoog's medium with coconut water and benzyl-adenine. They were able to produce a continuously proliferating tissue from banana shoot tips, previously irradiated with 1.0 Kr gamma radiation.

A study on the use of sugarcane bagasse as mulch for tomato showed that at four months after milling, the bagasse mulch induced chlorosis and stunting, indicating the presence of a water-soluble inhibitory substance. However, after 6-8 months, the mulch stimulated the growth of seedlings.

A few of the miscellaneous observations on growth regulators follow: In cassava, naphthalene acetic acid and indole butyric acid increased the yield of fleshy roots when cuttings were soaked in 100-150 ppm solution for 4 hours before planting (151). Basal cuttings of sweet potato have less endogenous growth regulators than terminal cuttings (162). In the field, sprays of 50 ppm indole-3 butyric acid increased the yield of basal cuttings (161). In Irish potato, coumarin inhibited production of dry matter in the roots, and delayed tuberization (156).

Under stress, plants produce substances which cause morphogenic abnormality. For example, tomato plants under water-logged conditions increased their abscissic acid content; produced ethylene and epinasty of leaf petiole. By adding di-iodo-4 hydroxybenzoic acid in the water-logging solution, Alejar (130) was able to decrease ethylene in the rhizosphere; improve root growth and reduce abscissic acid in the root and shoot. In rice, di-iodo-4 hydroxybenzoic acid induced root elongation, and dry-matter production in roots and shoot. It did not increase nitrogen uptake (131).

On the other hand, light and wind served as a stress on leafy vegetables, causing the loss of ascorbic acid (140).

### *Climate and growth; miscellaneous*

The genotype-environment interaction in soybean was studied by Salehuzzaman and Joarder (176). They planted twenty varieties every month from May to December. (Each planting was expected to differ from the others in the climatic environment it underwent.) They computed the analysis of variance for six characters, among which were height and weight of plants. The regression coefficient ( $b$ ) is a measure of the response to environment and the deviation from regression ( $S_d^{-2}$ ) is a measure of stability of the genotype. They concluded on the basis of ( $b$ ) and  $S_d^{-2}$  that improved Pelican, Biloxi and EC-11780 were suitable for growing in favorable environments. Variety TK-5 performed better in poor environment. Varieties Kao Asiang, Multor and Wells matured early in environments which tended to delay harvest.

Among the months of the year, the best time for growing jute and kenap is April to June. The April planting produced the tallest and biggest stalk; plants had the most dry fiber yield and most number of seeds (136).

Sorghum, a sun-loving plant, suffered markedly when shaded. Specific leaf weight, leaf area index, average height, total dry matter yield, total grain yield and grain-to-straw ratio decreased as shading was intensified. With decreasing light intensities, there was an increase in protein, but a decrease in carbohydrates (165). The callus tissue of sugarcane, another sun-loving plant, was grown in light intensities of

effect on differentiation. In Murasshigce and Skoog's medium, there was differentiation into shoot at 200 foot-candles and into root at 700. The 1,000 foot-candle intensity was not conducive to differentiation into shoot or root. On the other hand, after differentiation, high light intensity was beneficial. Plantlets exposed to 400 up to 1,000 foot candles had profusely branched roots, high percentage of dry matter and higher percentage of survival when transplanted to the soil (160). On the other hand, purslane (*Portulaca oleracea* L.), a shade-tolerant weed, grew to the same extent at 25 and 50% shading as in full sunlight. However, its growth habit changed with shading from the normal prostrate habit to vertical. At 75% shading, branching was less; also, fresh weight, flower and seed production were reduced (186).

Net carbon assimilation is used as a measure of net photosynthesis in the field. It is the resultant of photosynthesis, a carbon dioxide-consuming process, and of respiration, a  $\text{CO}_2$ -releasing process. Three sugarcane varieties, known to have different carbon exchange capacities, were found to have significant differences in leaf area, number of functional leaves, and base Brix reading (sugar concentration). Despite differences in leaf morphology (short vs long, narrow vs wide), total dry

weight of leaves did not vary significantly among varieties (170). In sorghum, the varieties differed in rates of photosynthesis. EG-6, a medium short cultivar with narrow erect leaves had the highest photosynthetic rate, while IS-295, a tall cultivar with long, wide and drooping leaves, had the lowest. Carbon dioxide assimilation was increased by higher light intensity and population density. Correlations among photosynthesis, night respiration and grain yield were highly significant.

Pollution of the Pasig River is an environmental stress on some aquatic forms. Tamayo-Zafaralla (181, 182) found that the composition, abundance and distribution of benthic algae differed between months of sampling. They reflected the changes in salinity, dissolved oxygen, orthophosphate and ammonia. Rains had a diluting and flushing effect on the pollutants, hence, an effect on the algae. Pantastico *et al.* (167) gave an enumeration of blue-green algae, found in various localities. This group is unique in being mainly found in polluted habitat, and in having the capacity to fix nitrogen.

The other studies concerned the induction of branching in coconut with wounding (133), yellowing (curing) of tobacco leaves as related with chlorophyll, abscissic acid, etc. (138), biochemical changes (dehydrogenase, decarboxylase, respiratory quotient) during deterioration in storage (158) and analysis of organophosphate insecticide residues in string beans (184).

### The Decade 1975-1984 in Retrospect

The workers in plant eco-physiology have been quite active during the decade. Their actual output is probably more than the 187 papers covered in this review; the writers could have inadvertently missed other papers in the less-widely available publications. Despite this volume of work, some people might still ask, "Yes, but how about the quality?" The writers' answer to this query, is that quality is usually a function of quantity. Thus, to place third among three competitors is not as much an accomplishment as being third best among 187 entries. However, while feeling felicitous about the amount of effort, the writers do not mean to create the impression that the output leaves no room for improvement. Wisdom or "wishdom" by hindsight can have a place in planning for the future.

Going over the list of papers, one will note that 47 studies were made on rice, 25 on coconut, 14 on maize or corn, 12 on sugarcane, 10 on soybean, 7 on tomato, 6 on cotton and 5 on Irish potato. Each of the other crops was treated in less than five papers.

The importance given each crop by the researchers did not strictly follow its place of importance in the field or in the market. Thus, they correctly gave importance to rice, coconut and corn, gauged by the area planted to these crops (Table 6); however, the importance given to rice was not in proportion to the area which it covered. Again, on the basis of area planted to the crop, root crops, sugarcane, banana and abaca should come next in the attention of the researchers. Except for sugarcane, they hardly touched those crops.

Table 6. Agricultural area per crop, amount and value of harvest in 1979 (cf, Philippine Statistical Yearbook, 1982)

<i>Crop</i>	<i>Area 1,000 ha</i>	<i>Harvest 1,000 m. tons</i>	<i>Value million pesos</i>
1. Total	11,968.7	28,597.7	₱33,758.3
2. Food		20,835.9	20,581.0
3. Commercial		7,761.8	13,177.3
1. Palay	3,468.9	7,197.6	6,925.2
2. Corn	3,326.9	3,167.4	2,934.7
3. Banana	327.8	4,179.0	1,994.3
4. Mango	38.6	363.3	1,056.5
5. Pineapple	54.6	604.6	736.1
6. Other fruits	69.4	607.7	1,055.5
7. Citrus	24.9	122.1	311.2
8. Root crops	480.7	3,568.8	1,562.9
9. Vegetables	67.7	467.2	992.3
10. Beans & peas	62.6	42.0	195.5
11. Coffee	95.2	115.5	1,755.0
12. Cacao	4.5	3.8	132.8
13. Peanuts	53.8	49.2	181.4
14. Other food crops	85.2	347.7	747.6
15. Coconut	2,994.6	4,295.4	8,524.9
16. Sugarcane	451.2	3,198.9	3,762.5
17. Abaca	234.7	148.3	343.0
18. Native tobacco	37.3	28.1	189.3
19. Virginia tobacco	29.6	23.2	151.0
20. Ramie	0.3	1.4	3.2
21. Rubber	53.7	58.8	190.1
22. Maguey	3.8	3.9	4.4
23. Other commercial crops	5.7	3.7	9.0

If the volume of business on the crop is used as a measure of importance, coconut, rice, sugarcane, corn, banana, coffee, root crops, mango and other fruits would merit the highest attention of our researchers. On the other hand, cacao, mango, coffee, pineapple, citrus and vegetables gave the highest value of product per hectare.

Of course, there should be some flexibility in relating research effort with the importance of the crop. Some of the complementary considerations are the state-of-the-art in the culture of the crop, its "tractability" for use as guinea pig, and the desired stance of the crop in the market (i.e., whether aggressive or defensive).

With the possible exception of the studies on soil of coconut groves affected by the cadang-cadang (blight) disease, the studies were an echo of studies per-

formed abroad. Most of them stopped at performance testing, and were silent on the broader objective. For example, in the study to compare herbicides, the researchers were silent on whether they expect the public to continue buying the imported chemical or they expect to influence its local manufacture.

Perhaps our researchers should pursue some locally generated concept, no matter how "queer" the thought might be. At least, this would be a step towards independent thinking. (The toddling steps of a child, although awkward, may develop into a sprint that could win a 100-meter race.) The writers would like to propose two moot examples of areas where a train of thought could be stimulated. They might attract more opponents than adherents; but if the examples could stimulate thinking on the subject they would have served their purpose:

1) People in over-populated countries (and confronting the specter of famine) are at a loss in producing their staple food — primarily starch. The grains (rice, corn, sorghum etc.) are less efficient starch-producers than the root crops such as cassava, gabi and camote. Should not the researchers study the latter group and attempt to make it a more effective substitute for the former? Secondly, being both field crops, the grains and the root crops require periodic land preparation, which is labor intensive. Orchard crops, on the other hand, do not require periodic land preparation. Should we not improve the culture of orchard crops like breadfruit, thiesa, mabolo, and zapote, (and semi-permanent crops like banana) to supplement the production of starch? The Japanese people were able to modify their eating habit so that they could afford to eat less rice. They meet part of their need for starch with supplements like root crops and seaweeds.

2) Towards exploiting the potentials of our environment to the utmost, should we locate at regular intervals research stations from Sulu to Batanes? Knowledge on the eco-physiological reactions of crops to latitude (i.e., temperature and day-length patterns) may help determine what crop to grow best in each region. Should we likewise disperse research stations from east to west of big landmasses (Luzon and Mindanao) to monitor the reaction of crops to rainfall patterns?

Of course, starch production and choice of a suitable crop for a given environment are but two areas in the broad field of eco-physiological studies. The other areas could be equally important, if not more. One excuse for this haphazard suggestion is the thought that (in focusing attention on a topic) we could gain in intensity of insight whatever we lose in scope of vision.

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# TAXONOMY OF PHILIPPINE DIPTERA\*

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## Introduction

The Diptera or true flies are insects with a pair of functional wings, except for a relatively few wingless forms. Other insects with only two wings are some species of mayflies or Ephemeroptera and male Coccoidea.

Dipterans have been a favorite subject of study because of their importance as human or animal pests, or as vectors of diseases. Among such pests and or vectors are mosquitoes, biting midges, blackflies, sandflies, houseflies, horseflies and blowflies. Mosquitoes transmit malaria, filariasis, and a number of viral diseases such as dengue, H-fever and encephalitides.

Many species such as fruitflies, leafminer flies and gall midges are pests of agricultural crops or forest trees. Others, however, are beneficial as parasites or predators that help regulate the populations of many plant and animal pests.

This paper attempts to present important taxonomic literature on Diptera described or recorded in the Philippines from 1758 to 1984, or a period of 226 years. A brief history of taxonomic studies on Philippine materials, including the preparation of Diptera catalogs, will be reviewed. In Table 1 is a classification scheme showing the diversity and taxonomic arrangement of families and higher categories, as well as the present count of genera and species under each family, and endemism expressed in percent. The predominant groups of Diptera in the Philippines are shown in Table 2 and the rare families, in Table 3. Table 4 presents the families of Diptera that are known in the Oriental region but are missing or unrecorded in the Philippines. A list of the dipterous families that need further studies is included.

## A Brief History of Taxonomic Studies on Philippine Diptera

### *Osten Sacken, C. R. and pre-Bezzi workers (1882-1912)*

Before Osten Sacken embarked on the study of Philippine Diptera in 1880, only 52 species of Diptera were known and these were described by Macquart (10 spp.), Walker (11 spp.), Bigot and Wiedeman (2 spp. each), and Westwood (1 sp.). Additional species were described by Schiner (6 spp.) and Thomson (19

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\*Paper presented at the *State-of-the-Art* Seminar Series of the Biological Sciences Division, National Academy of Science and Technology, held at the NAST Conference Room on December 18, 1985.

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Table 1. Summary of genera and species of Diptera in the Philippines  
(As of 1985)

	<i>Genera</i>	<i>Species &amp; Subspecies</i>	<i>Percent Endemism</i>
SUBORDER NEMATOCERA (15 Families)	(134)	1,080	
Superfamily TIPULOIDEA	(37)	(409)	
Family Tipulidae	37	409	95.8
Superfamily PSYCHODOIDEA	(13)	(126)	
Family Psychodidae	11	101	74.3
Family Phlebotomidae	2	25	96.0
Superfamily CULICOIDEA	(46)	(464)	
Family Blephariceridae	1	1	100.0
Family Chaoboridae	1	1	100.0
Family Culicidae	17	274	47.0
Family Ceratopogonidae	19	88	19.3
Family Chironomidae	7	12	75.0
Family Simuliidae	1	88	96.6
Superfamily ANISOPODOIDEA	(1)	(2)	
Family Anisopodidae	1	2	50.0
Superfamily BIBIONOIDEA	(4)	(25)	
Family Bibionidae	4	25	80.0
Superfamily MYCETOPHILOIDEA	(33)	(54)	
Family Mycetophilidae	7	9	44.4
Family Sciaridae	4	10	40.0
Family Scatopsidae	3	4	75.0
Family Cecidomyiidae	19	31	83.9
SUBORDER BRACHYCERA (13 Families)	(103)	(358)	
Superfamily TABANOIDEA	(35)	(132)	
Family Rachiceridae	2	4	100.0
Family Solvidae	1	4	25.0
Family Stratiomyidae	19	43	39.5
Family Tabanidae	6	61	72.0
Family Rhagionidae	7	20	80.0
Superfamily ASILOIDEA	(41)	(114)	
Family Therevidae	1	2	100.0
Family Mydidae	1	1	—
Family Asilidae	28	80	76.3
Family Nemestrinidae	1	1	100.0
Family Acroceridae	2	5	100.0
Family Bombyliidae	8	25	56.0

Table 1 continued

	<i>Genera</i>	<i>Species &amp; Subspecies</i>	<i>Percent Endemism</i>
Superfamily EMPIDOIDEA	(27)	(112)	
Family Empididae	10	42	81.0
Family Dolichopodidae	17	70	48.6
SUBORDER CYCLORRHAPHA (47 Families)	(457)	(1,358)	
Division ASCHIZA	(56)	(260)	
Superfamily PHOROIDEA	(16)	(107)	
Family Phoridae	14	105	82.0
Family Platypezidae	2	2	—
Superfamily SYRPHOIDEA	(40)	(153)	
Family Pipunculidae	3	49	49.0
Family Syrphidae	35	92	39.1
Family Conopidae	4	12	75.0
Division SCHZOPHORA			
Section ACALYPTRATE			
Superfamily MICROPEZOIDEA	(12)	(32)	
Family Cypselomatidae	1	1	—
Family Pseudopomyzidae	1	1	100.0
Family Neriidae	3	5	60.0
Family Micropezidae	7	15	60.0
Superfamily NOTHYBOIDEA	(9)	(21)	
Family Strongylophthalmyiidae	1	5	40.0
Family Psilidae	2	3	33.3
Family Megamerinidae	2	2	—
Family Nothybidae	1	1	—
Family Diopsidae	3	10	50.0
Superfamily OTITOIDEA	(81)	(243)	
Family Pyrgotidae	2	4	100.0
Family Tephritidae	54	164	62.2
Family Platystomatidae	23	73	63.0
Family Otitidae	2	2	50.0
Superfamily SCIOMYZOIDEA	(7)	(20)	
Family Sciomyzidae	2	7	—
Family Sepsidae	5	13	—
Superfamily LAUXANIOIDEA	(29)	(129)	
Family Lauxaniidae	24	97	65.9
Family Celyphidae	4	31	74.1
Family Chamaemyiidae	1	1	100.0

Table 1 continued

	<i>Genera</i>	<i>Species &amp; Subspecies</i>	<i>Percent Endemism</i>
Superfamily PALLOPTEROIDEA	(4)	(12)	
Family Lonchaeidae	3	11	18.1
Family Piophilidae	1	1	—
Superfamily ANTHOMYZOIDEA	(8)	(17)	
Family Aulacigastridae	2	2	—
Family Clusiidae	7	15	40.0
Superfamily AGROMYZOIDEA	(7)	(26)	
Family Agromyzidae	7	26	23.0
Superfamily ASTEIOIDEA	(1)	(1)	
Family Neurochaetidae	1	1	100.0
Superfamily CHLOROPOIDEA	(37)	(104)	
Family Milichiidae	2	8	12.5
Family Chloropidae	37	97	56.7
Superfamily DROSOPHILOIDEA	(34)	(96)	
Family Diastatidae	1	1	100.0
Family Ephydriidae	20	37	21.6
Family Curtonotidae	2	2	50.0
Family Drosophilidae	11	57	38.5
Systematic position not clear	(8)	(19)	
Family Canaceidae	3	4	—
Family Tethinidae	1	3	33.3
Family Sphaeroceridae	4	11	18.1
Section CALYPTRATAE			
Superfamily GLOSSINOIDEA	(21)	(57)	
Family Hippoboscidae	11	28	28.5
Family Nycteribiidae	7	21	42.8
Family Streblidae	3	8	50.0
Superfamily MUSCOIDEA	(34)	(112)	
Family Anthomyiidae	3	4	—
Family Muscidae	30	107	19.6
Family Egnidae	1	1	—
Superfamily CALLIPHOROIDEA	(109)	(212)	
Family Calliphoridae	19	51	35.2
Family Sacrophagidae	8	14	14.2
Family Tachinidae	82	146	42.4
<b>T O T A L</b>	<b>697</b>	<b>2,799</b>	<b>58.5</b>

Table 2. Superfamilies ranked by the greatest number of Diptera  
(As of 1985)

<i>Superfamily</i>	<i>No. of Species and Subspecies</i>	<i>Percent of total species</i>
1. CULICOIDEA (mosquitoes, gnats, blackflies)	464	16.59
2. TIPULOIDEA (craneflies)	409	14.62
3. OTTOIDEA (fruitflies)	243	8.69
4. CALLIPHOROIDEA (blue bottles, flesh flies, tachinid flies)	212	7.58
5. SYRPHOIDEA (flower flies or hover flies, big-eyed flies, thick-headed flies)	153	5.47
6. TABANOIDEA (horse flies, soldier flies, snipe flies)	132	4.72
7. LAUXANIOIDEA (lauxanid flies)	127	4.54
8. PSYCHODOIDEA (moth flies, sand flies)	126	4.51
9. ASILOIDEA (robber flies, stiletto flies, small- headed flies, bee flies)	114	4.08
10. MUSCOIDEA (houseflies)	112	4.01
11. EMPIDOIDEA (dance flies, long-legged flies)	112	4.01
12. PHOROIDEA (humped-back flies)	107	3.83
13. CHLOROPOIDEA (chloropid or frit flies)	104	3.72
14. DROSOPHILOIDEA (vinegar flies, pomace flies)	96	3.43
15. GLOSSINOIDEA (bat flies, louse flies)	57	2.04
16. MYCETOPHILOIDEA (fungus gnats, gall flies)	54	1.93
17. MICROPEZOIDEA (stilt-legged flies)	32	1.14
18. AGROMYZOIDEA (leafminer flies)	26	0.90
19. BIBIONOIDEA (March flies)	25	0.90
20. NOTHYBOIDEA (stalk-eyed flies, rust flies)	21	0.75
21. SCIOMYZOIDEA (marsh flies)	20	0.72
22. Unplaced families	19	0.68
23. ANTHOMOZOIDEA (frit flies)	17	0.61
24. PALLOPTEROIDEA (pallopterid flies)	12	0.43
25. ANISOPOIDEA	2	0.07
26. ASTEIOIDEA	1	0.03
	2,799	100.00

Table 3. Diptera families with only 1 to 5 species known in the Philippines compared with the total known in the Oriental region

	<i>Philippines</i>	<i>Oriental region</i>		<i>Philippines</i>	<i>Oriental region</i>
<b>NEMATOCERA:</b>			<b>CYCLORRHAPHA:</b>		
Blephariceridae	1	21	Cypselosomatidae	1	5
Scatopsidae	4	24	Pseudopomyzidae	1	1
<b>BRACHYCERA:</b>			Strongylophthalmyidae	5	22
Rachiceridae	4	40	Otitidae	2	12
Solvidae	4	46	Sciomyzidae	5	23
Therevidae	2	33	Chamaemyiidae	1	9
Mydidae	1	9	Piophilidae	1	3
Acroceridae	5	39	Neurochaetidae	1	2
			Aulacigastridae	1	3
			Diastatidae	1	3
			Canaceidae	1	18
			Tethinidae	3	7
			Anthomyiidae	4	50

Table 4. Diptera families found in the Oriental region but not represented in the Philippines (Ref.: *Catalog of Diptera of the Oriental region*, Vols. I to III and Baltazar's Catalog, in preparation)

	<i>Genera</i>	<i>Species</i>		<i>Genera</i>	<i>Species</i>
<b>NEMATOCERA:</b>			<b>CYCLORRHAPHA:</b>		
Trichoceridae	2	25	Micropezidae	10	51
Tanyderidae	1	3	Dryomyzidae	1	1
Ptychopteridae	2	16	Teratomyzidae	1	1
Nymphomyiidae	1	1	Asteiidae	3	11
Deuterophlebiidae	1	1	Chyromyidae	2	3
Dixidae	1	15	Anthomyzidae	2	2
Thaumaleidae	1	15	Oдиниidae	2	2
Anisopodidae	3	22	Carnidae	2	5
Pachyneuridae	1	1	Heleomyzidae	1	6
<b>BRACHYCERA:</b>			Heteromyzidae	1	2
Coenomyiidae	1	1	Cryptochetidae	1	7
Scenopinidae	3	10	Scathophagidae	9	4
Apioceridae	1	1	Fanniidae	2	30
Lonchopteridae	1	10	Eginiidae	8	9
Platypezidae	7	14	Rhinophoridae	4	4
			Gasterophilidae	3	6
			Oestridae	6	9

spp.) based on materials brought home by the naturalists of the "Novara" and the "Eugenia".

Osten Sacken was the first dipterologist to undertake a comprehensive study of the dipterous fauna of the Philippines. His study was based on materials collected by Dr. Carl Semper, a former professor of Wurzburg, Germany, who resided in the Philippines around 1859 to 1864. Semper's collection, consisting of about 250 species, was first sent to Prof. L. Bellardi in Turin, Italy, but he was not able to work on it; instead he sent the materials to Walker for comparison with the specimens in the British Museum. The Philippine species described by Walker were few in number and these were included in his 1866 "Synopsis of the Diptera of the Eastern Archipelago, discovered by Wallace." Finally, Semper's Diptera collection fell into the hands of Osten Sacken in 1880. The latter's significant work entitled "Diptera from the Philippine Islands brought home by Dr. Carl Semper" was published in two parts in *Berl. Ent. Ztschr.* (1882). He named 132 to the species level, with 24 others identified to the genus level, making a total of 156 species.

In Father Casto de Elera's "Catalogo sistemático de toda la fauna de Filipinas conocida hasta el presente" (1895), nothing was added to the Diptera list of Osten Sacken. In the family Culicidae, many species of mosquitoes were discovered and described by Giles, C. S. Banks, Ludlow, Knab and Theobald. Some new species of Phoridae were described by Brues (1909), Tabanidae by Ricardo (1911), Hippoboscidae by Speiser (1904-1905), Anthomyiidae by Stein (1900-1919).

J. C. H. de Meijere published in *Tijdschr. Ent.* (1907-1924) entitled "Studien ueber sudostasiatische Dipteren," I to XV; many of the Diptera species described from Southeast Asia extend their distribution to the Philippines.

#### *Bezzi, M. and pre-war taxonomists (1913-1945)*

Like Osten Sacken, Bezzi prepared a "Catalogue of the Diptera hitherto recorded from the Philippine Islands" (1913) before launching into his studies on Philippine Diptera, based on materials sent by Dr. C. F. Baker, former professor and dean of the U.P. College of Agriculture. This catalog listed 283 species with 6 additional species published up to 1914.

The first part of Bezzi's studies consisted of 100 species identified from Baker's collections in Luzon only. The second part was composed of another 100 species collected in other islands of the Philippines, including blood-sucking species sent by Mr. M.B. Mitzmain for identification. Bezzi described 55 new species and two new varieties; however, his original description of new species did not state the number of specimens in the type series, nor was there a type designation. Lectotypes were subsequently designated by Delfinado in 1969 when she studied what was left of the Bezzi Diptera collection in the Museo Civico di Storia Naturale in Milano, Italy. According to her, the material is well preserved and in good order.

After the publication of Bezzi's Diptera catalogue, two European workers made major contributions by describing many new species from G. Boettcher's materials that were collected around 1911 to 1918, mostly in Luzon, Masbate,

Mindoro, Mindanao and Palawan. F. W. Edwards of the British Museum (Natural History) published in three parts on "Philippine Nematocerous Diptera" (1926 and 1229). Richard Frey, on the other hand, published a series of articles in German entitled "Philippinische Dipteren", parts I (1923) to VIII (1930), composed of the Brachycera and Cyclorrhapha.

When Baker died in 1927, his Philippine collection (including those from Borneo and Singapore) was bequeathed to the U.S. National Museum in Washington, D. C. Although the exact locality and date of collection were not indicated on the specimens, nonetheless, the collection is invaluable as it has been the source of materials for taxonomic studies by many authors: Alexander (1913-1961) monopolized the study of Philippine Tipulidae except for a few species described by Edwards (1926). Cresson (1925-1948) published on Ephydriidae; Dyar (1920-1929) on mosquitoes; Felt (1915-1920) on gall midges or Cecidomyiidae; Ferris (1924-1927) on Diptera Pupipara; Kieffer (1922) on Chironomidae; Kroeber (1924-1940) on Conopidae, Tabanidae, and Therevidae; Malloch (1912-1939) on Phoridae, Celyphidae, Platystomatidae, Lauxaniidae, etc.; Sack (1926) on Syrphidae; Sturtevant (1927) on Drosophilidae; Townsend (1928) on Muscidae.

Del Rosario (1936) and Manalang (1930-1931) from the Philippines worked on Psychodidae; Mendoza (1940-1954), also from the Philippines, and Russel (1934-1936), and Bohart (1945) published on Culicidae.

Brues (1936) published on Philippine Phoridae based on material collected by C. F. Clagg who spent several months in the Mt. Apo region, Mindanao during 1930-1931. Clagg's collection was deposited in the Museum of Comparative Zoology, Harvard University, Massachusetts, U.S.A.

#### *Post-war taxonomists (1946-1970)*

Immediately after World War II, the Chicago Museum of Natural History sent a Philippine Zoological Expedition in 1946 to 1947 headed by Hoogstraal. In the late 50's and up to the 60's, the Department of Entomology of the Bernice P. Bishop Museum at Honolulu, Hawaii, headed by the late Dr. L. Gressitt, sent entomologists, namely: Drs. C. Milliron, Carl Yoshimoto, H. Hirashima, L. Quate and several others to collect in various islands in the Philippines, but especially in Palawan, Mindanao and Sulu Archipelago. These collections added new materials for study and a lot of Diptera were caught by Malaise traps.

During this period, many taxonomic papers dealt with Diptera of medical and veterinary importance. For example, contributors to the study of Philippine Culicidae are the following: Baisas (1946-1958, 1965), Delfinado (1966 to 1968), Knight (1946-1953), Laffoon (1946), Mattingly (1957-1970), and Rozeboom (1946-1966). The biting midges (Ceratopogonidae) and the black flies (Simuliidae) were studied by Delfinado (1961, 1962, 1969). The taxonomy of Philippine Psychodidae was done by Quate (1965). Philip (1959) published on Tabanidae. Work-

ers on Diptera Pupipara (Hippoboscidae, Nycteribiidae and Streblidae) are Maa (1962-1969) and Theodor (1953-1963).

Other taxonomic studies that dealt with various families and their corresponding contributors are: Beyer (1960, 1966) and Borgmeier (1967) on Phoridae; Das Gupta and Wirth (1968) and Saunders (1964) on Ceratopogonidae; Nagatomi (1970) on Rachiceridae; Parent (1941) on Indo-Australian Dolichopodidae; Sasaki (1963) on Agromyzidae; Tenorio (1963, 1969) on Celyphidae; Wirth (1964) on Canaceidae and Ephydriidae; Yano (1968) on Sciomyzidae.

Studies based largely on the Danish Noona Dan Expedition (1961-1962) to the southern Philippines and the Bismarck Islands, supplemented by loan materials from the United States and other European museums, resulted in several publications authored by the following: Bowden (1971) on Bombyliidae; Chvala and Lyneborg (1970) on Tabanidae; Delfinado (1971) on some Simuliidae and Curtonotidae; Hardy (1968) on Bibionidae and Pipunculidae, Hardy (1970) on Tephritidae; James (1966) on blowflies or Calliphoridae, James (1969) on soldier flies or Stratiomyidae; Lopes (1967) on Sarcophagidae; Pont (1968) on Muscidae; Sasaki (1974) on Clusiidae; Spencer (1965) on Agromyzidae; Theodor (1966) on Diptera Pupipara; Vanschuytbroeck (1967) on Celyphidae.

*Delfinado, Hardy and recent workers (1971 to the present, 1985).*

The publication of the Catalog of the Diptera of the Oriental Region, Volumes 1 to 3 (1973, 1975 and 1977, respectively) by Diptera specialists throughout the world (edited by Delfinado and Hardy) paved the way for many revisional studies that followed.

Great advances in the taxonomy of the Culicidae consisted of revisions or contributions to the mosquito fauna of Southeast Asia published by Abercrombie (1977), Huang (1972-1979), Klein (1973), Mattingly (1970-1981), Peyton (1972, 1977), Reinert (1970-1985), Sirivanakarn (1971-1977), Tyson (1970), and Zavorintk (1971). A second edition of the Catalog of the mosquitoes of the world was published by Knight and Stone (1977). Two supplements to the above-mentioned catalog were prepared by Knight (1978) and Ward (1984). Mosquito studies were supported mostly by the Southeast Asia Mosquito Project (SEAMP) of the Smithsonian Institution, Washington, D. C. and the U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland.

Recently, Miyagi *et al.* (1980-1983) described two new species of mosquitoes and conducted a survey of the mosquito fauna in Palawan, Mindanao and Northern Luzon (1985). Basio (1971) monographed the mosquito fauna of the Philippines. A new list of Philippine mosquitoes was compiled by Cagampang-Ramos *et al.* (1985).

Other noteworthy contributions after the Oriental Diptera Catalog was published are the following: Baisas (1974) on the mosquito fauna of Subic Bay; Burger and Thomson (1981) on the *Tabanus striatus* complex; Crosskey (1976) on Talchinidae of the Oriental Region, also Dear and Crosskey (1982) on Phil-

ippine Tachinidae; Cuy (1980, 1981) on Philippine Nycteribiidae and Strebliidae; Evenhuis and Arakaki (1980) on Philippine Bombyliidae; Hardy (1974) on the fruit flies (Tephritidae) of the Philippines; Hull (1973) on Bee flies (Bombyliidae) of the World; Mattingly (1971, 1981) on Culicidae; Moucha (1976) on the horse flies (Tabanidae) of the world: synoptic catalog; Okada (1977 to 1985) on Drosophilidae; Oldroyd (1972) on robber flies (Asilidae) of the Philippine Islands; Philip (1974-1981) on Oriental Tabanidae; Rueda (1985) on some Philippine blowflies (Calliphoridae); Sabrosky (1983) on world species of *Desmometopa* (Milichiidae); Takaoka (1983) on the black flies (Simuliidae) of the Philippines.

## Important Revisions and References on Specific Groups of Diptera

### Suborder NEMATOCERA

#### *Tipulidae:*

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#### *Psychodidae:*

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- Quate, L. W. 1965. A taxonomic study of Philippine Psychodidae (Diptera). *Pacif. Insects* 7: 815-902.

#### *Culicidae:*

### List of Catalogs

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#### *Anophelinae:*

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#### *Toxorhynchitinae:*

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#### *Culicinae:*

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**Suggestions**

Taxonomic researches on the following important families of Diptera that are least studied should be undertaken:

*Suborder Nematocera:*

- a. Chironomidae (midges) – The only available reference on this group of insects was that published by Kieffer (1925). Very many species of midges are found in the Philippines and since they are important as fish food, the family should be studied.
- b. Cecidomyiidae (gall gnats) – This is another group of flies that is a good subject for study. The last publication on Philippine cecidomyiids was in 1925 by Felt.
- c. Mycetophilidae (fungus gnats) and Sciaridae – These gnats are common in moist habitats and forest undergrowth. Although these are large families, they are poorly studied in the Philippines.
- d. Tipulidae (crane flies) – While there are over 400 species in the Philippines, described mostly by Alexander, there is no available key to use for identifying these species. Alexander's types at the Smithsonian Institution should be studied. The biology and immature stages of these flies are hardly known here.

*Suborder Brachycera:*

- a. Dolichopodidae (long-legged flies) – This family is well represented in the Philippines but poorly studied. Becker's monograph (1922) and Frey's work (1924 & 1925) are the only references available on Philippine species.

*Suborder Cyclorrhapha:*

- a. Syrphidae (Hover or Flower flies) – Very many species of syrphids are found in the Philippines. A comprehensive study is badly needed, considering that work on Philippine species was done by Sack in 1926 and Hull in 1937, 1944 and 1950. These flies are important as pollinators in the adult stage and as natural enemies of aphids, scale insects and soft-bodied insects in the larval stage.
- b. Diopsidae (stalk-eyed flies) – These are small- to medium-sized flies commonly found in forest undergrowth and moist habitats. Only nine species

have been described. A serious study of the Philippine species has yet to be done.

c. Agromyzidae (leafminer flies) – The larvae of these small flies are leaf-miners, stem-borers or miners, and root or seed-feeders. No comprehensive study of the Philippine species has been done.

d. Chloropidae (frit flies, “eye fly”) – Frey’s publication (1923) is about the only work on Philippine species. More studies are needed.

e. Ephydriidae (shore flies) – Cresson’s papers (1945 & 1948) are the most recent work on Indo-Australian Ephydriidae. An up-date is necessary for this beneficial group of flies that serve as food for wildlife especially in marshy areas.

f. Drosophilidae (pomace flies) – Although this family is very abundant in species, no monographic study has been done.

g. Muscoidea (Muscidae, Calliphoridae and Sarcophagidae) – Despite the medico-veterinary importance of this group of flies, and their extreme abundance in the Philippines, no comprehensive study has been done. Rueda (1980) did a taxonomic study of the calliphorids for his M.S. thesis at U.P. Los Baños and this has been published recently (1985). A handbook on the Muscoidea of the Philippines is badly needed.



# FISH PARASITOLOGY AND AQUACULTURE MANAGEMENT IN THE PHILIPPINES\*

Carmen C. Velasquez\*\*

## ABSTRACT

Herein presented is the status of fish parasites and diseases research in the Philippines. Limitations in fish health management and productivity in fish culture are discussed. Diseases communicable or potentially communicable to man via fish or the water are included. Areas requiring further research are noted.

## Introduction

In the promotion of the nation's economy, the country has turned to the use of all available resources. Fisheries, in particular aquaculture, is destined to play a role of increased importance in feeding our rapidly growing population.

The increasing operational costs of capture fishing and reported declining catches from our natural waters require intensified efforts to meet our country's protein needs. Aquaculture activities have resulted in the use of our marine and inland resources and in the importation of fishes for cultivation and propagation in our fishponds.

Our culture systems now include freshwater, brackishwater and marine species not only in ponds, but also in tanks, pens, cages, reservoirs, rice paddies, and even an entire lake. Most recently, integrated animal-fish, rice-fish farms, and coastal areas are now a part of the intensification program. The major fish and shellfish species cultured are *Chanos chanos* (bañig), *Tilapia* spp., *Clarias* spp., *Cyprinids* spp., *Penaeus monodon*, *Crassostrea* spp. *Mytilus* spp. Others include groupers, sea basses, *Macrobrachium* spp., *Scylla serrata*, squids and several species of shrimps.

Extensive and intensified activities suffer from problems of pests and diseases accompanied by intranational and international transfaunation.

Fish parasitology, like all phases of parasitology, embodies the study of relationships between the parasite and its abiotic or biotic environment. These

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relationships are multiple and complex, and the requirements for adaptation in each are different.

The general principles governing the changes that occur in fish-parasite fauna resulting from acclimatization in a new environment are now known. The disease of fishpond-bred fishes differ from those inhabiting natural environments. The management methods of pond fisheries favor infestations with fish parasites which when present in natural waters do not become economically important under existing environmental conditions. On the other hand, pond fishes allow either the control of the parasitic diseases or in some cases their prevention with proper fish health management.

Available data on the parasite fauna of acclimatized fishes have given information on the changes caused in the process of transfer and acclimatization. The character and extent of these often depend on the characteristics of the new environment and the way in which the fish has been transferred. The transfer brings the fish into an environment differing from the natural waters in its ichthyofauna, plankton, benthos with different hydrochemistry, temperature, etc. Changes in the composition of the parasite fauna reflect current environmental conditions.

Bodies of inland waters such as lakes, ponds, streams, rivers and swamps can be characterized by their parasite fauna in the same manner as by hydrobiological and other biological data.

In planning and constructing pond farms and fish nurseries, important considerations are (1) independent supply and drainage of water for spawning, nursery and quarantine ponds, (2) adequate water supply, and (3) observance of sanitation in all ponds.

The composition and changes in the parasite fauna of all fishes depend on (1) geographical location, (2) habitat, (3) season of the year, (4) type of bottom, (5) water quality and other abiotic and biotic factors in the environment.

The mode of feeding, diet, and morphology are important aspects in the biology of the host for the prevention, control and treatment of parasitic diseases. The nature of the external tissues, such as the character of scales and the thickness of the subcutaneous connective tissue layers, influence the ectoparasite fauna and the penetration of the skin by endoparasites. The structure and histology of the various parts of the digestive tract and other internal organs and digestive secretions play a role in the infestation of various parasites. Parasites feed either on the digestive contents of the host's intestine or the host's tissues or by osmotic absorption.

The food of the fish host may include various animals which serve as intermediate hosts for the completion of the life cycle. Physiological activities such as nutrition, prolonged starvation and other habits and of course man's activities influence the host and its parasitic fauna.

Parasites can affect the body of fish by (1) mechanical, (2) nutritional and other indirect means.

The complexities of the life cycle of certain species of parasites are constraints in finding the vulnerable points in their life. Knowledge of the life cycle is fundamental in the control, if not eradication, of the parasite.

Earlier efforts were purely academic and as such approached without having thought of their merits and practical applications to aquaculture.

Included in Tubangui's (1947) checklist are 3 species of Monogenea in 2 families, 26 species of Digenea in 9 families and 1 subfamily, 1 Cestoda in one family, 2 nematodes in 2 families and 2 species of Acanthocephala in 1 family from 22 genera and 24 species of fresh, brackishwater and marine fishes (Table 1). Unfortunately, all type specimens were destroyed during World War II.

The activities were mainly directed to the concept of parasitocoenosis applicable especially to the medical and veterinary fields.

Microbial infections were reported in connection with fish food poisoning.

Problems of pathogenicity and danger of fish health and sanitation have posed problems, some of which involve economic losses.

This paper draws attention to the factual materials related to fish health management and problems of public health significance. Representatives of major groups of parasites of fish cultured or with aquaculture potential in the Philippines are presented. Brief life cycles and their biology are discussed when information is available.

## Current Research Activities

### *Microbial Infections*

The report of Po *et al.* (1982) (Table 2) gives existing knowledge of fish diseases caused by microorganisms in this country.

### *Viral Infections*

Out of three listed, the only specifically identified viral causative agent of disease is that in *Penaeus monodon* and 2 unidentified, one in *Clarias macrocephalus* and the other in marketable siganids.

### *Bacterial Infections*

Pathological effects on tissues especially those of the skin, fins, kidneys and even the eye have been observed, causing mortalities of *Penaeus monodon* larvae, *Chanos chanos* fry and fingerlings, *Anguilla* sp. (eel), *Clarias macrocephalus* fingerlings, *Tilapia mossambica*, *T. zilli* and *Saretherodon niloticus* (= *T. nilotica*). Out of the 7 causative agents listed, only 2 have been specifically identified, 4 generically and 1 as Gram-negative bacilli.

### Fungal Infections

Only 4 fungi have been recorded, 2 specifically identified, 1 generically and 1 unidentified. Internal tissue damage in *P. monodon*, *P. indicus* larval stages, crab eggs and larvae and vascularization of both eyes in *Chanos chanos* were observed due to fungal growth.

### Protozoal Infections

The list includes 3 species of *Trichodina* and *Trichodinella* from the gills, fins and bodies of *Tilapia zilli*, *T. mossambica*, *Trichogaster trichopterus*, *Cyprinus carpio* and *Chanos chanos*; *Ichthyophthirius* spp. on eel and *Amyloodinium*-like (which is indefinitely identified) causing tissue damage to *Mugil cephalus*. The rest in the list, although found in *P. monodon* juveniles, have not been observed in fish kills (Table 3).

Epizootics of trichodiniasis caused by *Trichodina* sp. occur most frequently in young fish inhabiting nursery ponds; hence, prophylactic measures should be observed, such as good flow of water, not too shallow ponds and use of live food. Parent spawner should be bathed before being placed in the pond or removed soon after spawning. Therapeutic baths recommended are 2% solution of sodium chloride (NaCl) for 10-20 minutes, depending upon the age of the fish or 0.01% potassium permanganate solution.

The life cycle of *Ichthyophthirius*, as known, is presented in Fig. 1. It can live outside the host for at most 3 or 4 days. The optimum temperature for reproduction and development is 25-27°C. No reliable treatment has been found for

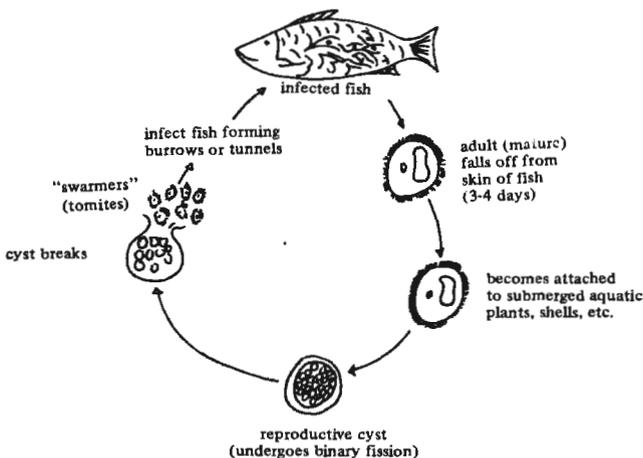


Fig. 1. Life cycle of *Ichthyophthirius multifiliis*.

Table 1. Parasitic helminths of Philippine fishes included in Tubangui's (1947) checklist

Parasite	Stage	Location	Host Species	Common Name	Habitat	Locality
<b>TREMATODA</b>						
<b>Monogenea</b>						
Dactylogyridae Bychowsky, 1933						
<i>Ancyrocephalus malienensis</i> Tubangui, 1931	adult and larva	gills	<i>Amphicentrus virgatus</i> syn. of <i>Tenthia virgata</i>	"samara" white-lined grouper	marine	Manila, Luzon Island
<i>Tetradium lutanii</i> Tubangui, 1931	adult and larva	gills	<i>Ausporodon leucogrammicus</i> <i>Lutjanus</i> sp.	"bam bañgin" "bam bañgin"	marine	Manila, Luzon Island
<i>Microcotyle virgaterum</i> Tubangui, 1931	adult and larva	gills	<i>Amphicentrus virgatus</i>	"samara"	marine	Manila, Luzon Island
<b>Digenes</b>						
<b>Bucephalidae Pochs, 1907</b>						
<i>Proporitynehus triangulatus</i> Tubangui and Maslufigan, 1944	adult	intestine	<i>Glossogobius giuris</i>	"biang puti"	freshwater	Manila, Luzon Island
<b>Clinostomidae (Luehe, 1901) Odhner, 1902</b>						
<i>Clinostomum dalesi</i> Tubangui, 1933	metacercaria	eye sockets	<i>Ophicephalus striatus</i>	"dalag"	freshwater	Los Baños, Laguna
<i>Clinostomum ophicephali</i> Tubangui and Maslufigan, 1944	metacercaria	gill bladder	<i>Ophicephalus striatus</i>	"dalag"	freshwater	Manila, Luzon Island
<i>Euclystosomum multicaecum</i> Tubangui and Maslufigan, 1935	metacercaria	muscles	<i>Ophicephalus striatus</i>	"dalag"	freshwater	Baso, Camarines Sur, Luzon
<b>Microcephalidae Travassos, 1922</b>						
<i>Hexangium affinis</i> Tubangui and Maslufigan, 1944	adult	intestine	<i>Amphicentrus javus</i>	"samara"	marine	Manila, Luzon
<b>Echinostomatidae Loois, 1902</b>						
<i>Echinostomum novaezelandense</i> Tubangui, 1932	metacercaria	muscles	<i>Glossogobius giuris</i> <i>Therapon plumbeus</i> <i>Ophicephalus striatus</i>	"biang puti" "ayulgin" "bangayangay"	freshwater freshwater freshwater	Manila Novaleses, Luzon Island Rizal, Luzon Island
<b>Allocrenidae (Stossich, 1904)</b>						
<i>Orientocreadium batrachoides</i> Tubangui, 1931	adult	intestine	<i>Clarias batrachus</i>	"hito"	freshwater	Los Baños, Laguna
<b>Heterophyidae</b>						
<i>Heterophyus expectans</i> (Africa and Garcia, 1935) Tubangui and Africa, 1937	metacercaria adult (dog and bird)	muscles	<i>Gerres kapei</i> <i>Gerres filamentosus</i> <i>Pelteus quadrilineatus</i> <i>Ambassis buruensis</i> <i>Hemiramphus georgii</i> Mugil sp.	"malakapas" "malakapas" "ba band" "langaray" "kanasait" "aligasin, banak" "iget"	marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater	Manila Obando Bulacan Luzon Luzon Luzon Palo, Leyte
<i>Galactosomum anguillarum</i> (Tubangui, 1933) Tubangui and Africa, 1938	adult	intestine	<i>Anguilla marmorata</i>	"igat"	marine, brackishwater	Manila Luzon
<i>Dioctotrema pseudocrotacei</i> Witenberg, 1929	metacercaria	muscles	<i>Ambas testudineus</i> Mugil sp.	"livalo" "aligasin, banak" "langaray" "kanasait"	marine, freshwater, marine, freshwater, marine, brackishwater freshwater, marine, brackishwater	Manila Luzon Bifan, Laguna
<i>Stictodora guerrei</i> Garcia and Refuerzo, 1936	metacercaria adult (cat, dog and bird, <i>Larus ridibundus</i> )	muscles	<i>Ambassis buruensis</i> <i>Hemiramphus georgii</i>	"langaray" "malakapas" "ba band" "sachos"	marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater	Luzon Manila Luzon Luzon
<i>Stictodora mantensis</i> Africa and Garcia, 1935	metacercaria adult (cat, dog and bird)	muscles	<i>Ambassis buruensis</i> <i>Gerres filamentosus</i> <i>Pelteus quadrilineatus</i> <i>Sillago sihama</i>	"langaray" "malakapas" "ba band" "sachos"	marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater	Luzon Manila Luzon Luzon
<i>Stictodora palmifera</i> Vasquez-Colet, 1943	metacercaria adult (dog)	muscles	<i>Ambassis buruensis</i>	"langaray"	marine, brackishwater	Luzon
<i>Microstromum</i> sp. Vasquez-Colet and Africa, 1938	metacercaria adult (cat, experimental infection)	muscle intestine	<i>Ambassis buruensis</i>	"langaray"	marine, brackishwater	Luzon
<b>Centrocestus formosanus</b> (Nishigori, 1924)						
<i>Centrocestus formosanus</i> (Nishigori, 1924)	metacercaria adult - bird ( <i>Bubulcus ibis coromandus</i> , <i>Ardea purpurea malienensis</i> ), also cat and dog (experimental infections)	muscles	<i>Ambas testudineus</i> <i>Glossogobius giuris</i> <i>Ophicephalus striatus</i> <i>Therapon plumbeus</i>	"livalo" "biang puti" "dalag" "ayulgin"	freshwater freshwater freshwater marine, brackishwater	Luzon Luzon Luzon Luzon
<i>Pygidioptis genata</i> Loois, 1907	metacercaria adult - cat and dog (experimental infection)	intestine muscles	Mugil sp.	"tallong, banak, aligasin"	marine, brackishwater	Luzon
<i>Pygidioptis maritima</i> Refuerzo and Garcia, 1937	metacercaria adult - bird	intestine muscles	Mugil sp.	"tallong, banak,	marine, brackishwater	Luzon

			<i>Amphicentrus javus</i> <i>Pelates quadrilineatus</i> <i>Eleutheronema tetradactyle</i> <i>Hemirhamphus georgii</i> <i>Ambassis buruensis</i> <i>Atherina babuacensis</i> <i>Mugil</i> sp.	"mamani" "babani" "mamani" "kasusuati" "lagarany" "guno" "tallong, banak, aligadin" "dalag, bulig" "biyang puti" "syungin" "malakapas" "amarsi"	marine marine marine, brackishwater marine, brackishwater marine, brackishwater marine, brackishwater	Northern Luzon
<i>Haplorchis pumilio</i> (Loos, 1896) Loos, 1899	metacercaria	muscles	<i>Ophicephalus striatus</i> <i>Glossogobius giuris</i> <i>Therapon plumbeus</i> <i>Ceres filamentosus</i> <i>Amphicentrus javus</i>		freshwater freshwater freshwater marine, brackishwater marine	
<i>Haplorchis stanoi</i> Africa, 1938	adult - cat, dog metacercaria	intestine muscles	<i>Ambassis buruensis</i> <i>Therapon plumbeus</i>	"lagarany" "syungin"	marine freshwater	Manila
<i>Haplorchis taiichui</i> (Nishigori, 1924) Chen, 1936	metacercaria adult - cat, dog, bird <i>Bubalucus ibite coromendus</i> and man	intestine muscles intestine	<i>Ophicephalus striatus</i>	"dalag, bulig"	freshwater	Manila, Leyte
<i>Haplorchis yokogawai</i> (Katsuma, 1932) Chen, 1936	metacercaria adult - man, cat, dog, bird, <i>Bubalucus ibite coromendus</i> , <i>Sirus whitsonii</i> and <i>Ardea purpurea</i> nestlings, monkey, <i>Pithecus mantidensis</i> (experimental infection)	muscles intestine	<i>Ophicephalus striatus</i> <i>Ceres lapis</i> <i>Amphicentrus javus</i> <i>Ambassis buruensis</i> <i>Hemirhamphus georgii</i> <i>Mugil</i> sp.	"dalag, bulig" "malakapas" "amarsi" "lagarany" "kasusuati" "banak, tallong"	freshwater marine marine brackishwater brackishwater brackishwater	Manila Manila Luzon
<b>Neohasmatidae</b>						
<i>Neohasmatium micromeris</i> (Tubangui, 1928) Tubangui and Masiliugan, 1944	adult	intestine	<i>Glossogobius giuris</i>	"biang puti"	freshwater	Manila Los Baños, Laguna
<b>Hemirhamphidae</b>						
<i>Ectenurus lemerletii</i> Tubangui and Masiliugan, 1935	adult	intestine	<i>Glossogobius giuris</i> <i>Scomberoides</i> sp.	"biang puti" "dorado, lapla"	freshwater marine	Manila and Balayan, Batangas Luzon
<b>Axyglidae</b>						
<i>Axygia pratripomae</i> Tubangui, 1928	adult	intestine	<i>Pomadasys haasi</i> <i>Glossogobius giuris</i>	"kiskisan" "biang puti"	marine, brackishwater freshwater	Manila Los Baños, Laguna Luzon
<b>Opecoelidae</b>						
<i>Opecoelus minimus</i> Tubangui 1928) Tubangui and Masiliugan 1944	adult	intestine	<i>Glossogobius giuris</i> <i>Ophicephalus striatus</i> <i>Therapon argenteus</i> <i>Chelodactylus batrachus</i> <i>Arilus mandentis</i>	"biang puti" "bulig, dalag" "arad-ad" "hito" "kandull"	freshwater freshwater marine, brackishwater freshwater freshwater	Manila Laguna de Bay, Luzon
<b>CESTODA</b>						
<b>Bothriocephalidae</b>						
<i>Bothriocephalus trevassoui</i> Tubangui, 1938	adult	intestine	<i>Anguilla marmorata</i>	"lasi"	marine	Palo, Leyte
<b>ACANTHOCEPHALA</b>						
<b>Rhadinorhynchidae</b> Travassos, 1923						
<i>Filicoma risulturnum</i> Tubangui and Masiliugan, 1946 <i>Neorhadinorhynchus octonucleatus</i> Tubangui, 1933	adult adult	intestine	<i>Scotophagus argus</i> <i>Therapon argenteus</i>	"kitang" "arad-ad"	freshwater, brackishwater marine, brackishwater	Malabon, Rizal Los Baños, Laguna
<b>NEMATODA</b>						
<b>Gnathostomatidae</b>						
<i>Gnathostoma spinigerum</i> Owen, 1876	larva-1st intermediate host - <i>Cyclops</i> ( <i>Eucyclops</i> ) <i>annulatus</i> and <i>C. (Microcyclops) bicolor</i> adult - dog, cat and civet cat, <i>Pseudoceros philippinensis</i>	muscles intestine	<i>Glossogobius giuris</i> <i>Ophicephalus striatus</i> <i>Therapon argenteus</i>	"biang puti" "dalag, bulig" "arad-ad"	freshwater freshwater	probably widely distributed probably widely distributed

Table 2. Microbial agents of fish and shellfish diseases reported in the Philippines (G.L. Po *et al.*, 1982)

<i>Agents</i>	<i>Host Species</i>	<i>Effects</i>	<i>Treatment</i>
<b>VIRUS</b>			
<i>Baculovirus</i> (MBV) unidentified	<i>Penaeus monodon</i> <i>Clarias macrocephalus</i> fingerlings	Hepatopancreas inflammation or sloughing of skin, fins, abdominal swelling and blood filled kidney	
unidentified	marketable siganids	lymphocystis	
<b>BACTERIA</b>			
<i>Aeromonas</i> sp.	<i>Clarias macrocephalus</i> Eel ( <i>Anguilla</i> sp.)	red spots on the belly, anal swelling and congestion at the base of the pectoral fins; high mortalities	Tetracycline or sulfamethoxine effective Erythromycin, sulfa drugs, furanace methylene blue for prophylaxis
<i>Beneckeia</i> sp.	<i>Chanos chanos</i> hatchery-reared and wild fry and fingerlings	sporadic mass mortalities	
<i>Flexibacter columnaris</i> (Myxobacteria) (isolated from kidney)	<i>Clarias macrocephalus</i> fingerlings and pre- adult (history of stress from excessive handling, crowding in cages and poor nutrition)	saddle-shaped lesions, gray, necrotic centers and inflamed borders, necrosis and erosions of skin with crater-like excavations into inflamed muscle tissues, and partial and complete erosion of fins and caudal peduncle; 90% kill after stocking; 80% on Tanay, Rizal seed production project	Furanace – inconclusive results sulfamonomethoxine daily 50-200 mg/kg fish or tetracycline daily 20-100 mg/kg fish in feed for 3-7 days severely affected fish did not feed
	Eel ( <i>Anguilla</i> sp.)	gray to white patches on skin, sloughing of affected skin and degeneration of gills; 10% mortalities	
Gram-negative bacilli	<i>Chanos chanos</i> <i>Clarias macrocephalus</i>	opaque eye dropsy-like	
Duncan, 1977			
<i>Trichodinella tilapiae</i> Duncan, 1977	<i>Tilapia zilli</i>	gills	
<i>Trichodinella</i> sp.	<i>Chanos chanos</i>	gills	ditto



<i>Haplochromis stasoni</i> Africa, 1938	metacercaria adult - cat and dog (experimental infection)	muscles	<i>Ambassia buruensis</i> <i>Therapon plumbeus</i>	"laAgaray" "ayuhgin"	marine freshwater	Manila
<i>Haplochromis taichui</i> (Nishigori, 1924) Chen, 1936	metacercaria adult - cat, dog, bird <i>Bubalcus ibis</i> coromander and man	intestinal muscles intestine	<i>Ophicephalus striatus</i>	"dalag, bulig"	freshwater	Manila, Leyte
<i>Haplochromis yokogawai</i> (Katsuta, 1932) Chen, 1936	metacercaria catfish	muscles	<i>Ophicephalus striatus</i>			

lesions at sites of attachment, fin inflammation

### *Tilapia mossambica*

pitechial hemorrhage, heavy pigmentation of skin, erosions of skin as well as fins and abnormal behavior; died 15% daily almost all stocks were lost  
25-100% of body covered; also gills at times  
17-50% mortalities

### *Tilapia zilli*

increased pigmentation, wasting lesions, surface swimming and anorexia  
red or inflamed fins and hemorrhagic spot on abdomen, lesions on surface of skin and fins-white spots  
lethargic; 90% mortality

Oxytetracycline and sulfonamides

### *Leucotrix* sp.

*P. monodon* post larvae

### *Pseudomonas fluorescens*

(very hardy, can survive various depths of water sources for more than 150 days)

*Sarotherodon niloticus* (*Tilapia nilotica*)  
(2 wk old tank reared)

Eel (*Anguilla* sp.)

inconclusive pathogenicity  
fin inflammation or erosions; pond mortalities in fish pens; loss 100,000 milkfish fingerlings

### *Vibrio* sp.

### *Vibrio* sp.

unidentified

*P. monodon* 1st to 3rd zoeal stage  
*Chanos chanos* (hatchery-reared and wild fry)

*Chanos chanos*

## FUNGI

### *Haliphthoros philippinensis*

### *Lagenidium* sp.

*P. monodon* larvae  
*P. monodon*  
*P. indicus* (naupliar, zoeal, mysis, post larval stages)

loss serious than *Lagenidium* infection  
internal tissues affected

Trifluralin - 0.005 ppm effective for zoospore development but not vesicle formation; 0.01 ppm effective for zoea and mysis

### *Lagenidium scylla*

crabs eggs and larvae (*Scylla serrata*)

pathological manifestations similar to that of penaeid larvae  
vascularization of one or both eyes; mortality 8% after 3 weeks

Treflan 1-5ppm effective

unidentified

*Chanos chanos*

Table 3. Protozoan agents of fish and shellfish diseases reported in the Philippines

<i>Agents</i>	<i>Host Species</i>	<i>Effects/site of infection</i>	<i>Reference</i>
<i>Amyloodinium</i> -like	<i>Mugil cephalus</i>	gill lesions and lamellar disintegration	G. L. Po <i>et al.</i> , 1982
<i>Ichthyophthirius</i> sp.	Eel ( <i>Anguilla</i> sp.)	surface skin lesions and fins -white spot disease; 20% mortality	↓
<i>Trichodina</i> spp.	<i>Sarotherodon niloticus</i> fry	high mortalities	ditto
<i>Trichodina acuta</i> Lom, 1961	<i>Tilapia zilli</i> <i>Tilapia mossambica</i>	gills, fins and body	Duncan, 1977
* <i>Trichodina heterodentata</i> Duncan, 1977	Population A <i>Tilapia mossambica</i> Population B <i>Tilapia mossambica</i> Population C <i>Trichogaster trichopterus</i>	body and fins gills, fins and body skin or body	↓
<i>Trichodinella carpi</i> Duncan, 1977	<i>Cyprinus carpio</i>	gills	↓
<i>Trichodinella tilapiae</i> Duncan, 1977	<i>Tilapia zilli</i>	gills	↓
<i>Trichodinella</i> sp.	<i>Chanos chanos</i>	gills	ditto

Table 3 continued

<i>Agents</i>	<i>Host Species</i>	<i>Effects/site of infection</i>	<i>Reference</i>
<i>Epistylis</i> sp.	<i>Macrobrachium rosenbergii</i>	suspected cause of mortalities mortality 70% resulting from decomposition of frog's meat used as feed not associated with fish kills	G. L. Po <i>et al.</i> , 1982
<i>Zoothamnium</i> sp.			↓
<i>Costia</i> sp.	<i>P. monodon</i> larvae		ditto
<i>Cryptobia</i> sp.			
<i>Ephelota gemmipara</i>			
<i>Gregarina</i>			
<i>Myzosoma</i> sp.			
<i>Scyphidia</i> sp.			
<i>Vorticella</i> sp.			

\*all 3 populations differed markedly in cell size and denticle shape, particularly in the frequency with which the characteristic central prominence occurred (Duncan, 1977).

ichthyophthiriasis. Maintenance of low density of fish population of the nursery and feeding ponds prevents the spread of the disease in affected farms.

Transmission of microbial pathogens depends on environmental conditions coupled with release from the host at varying intervals and may be enhanced if they breed freely in the water.

Microbial infections, communicable or potentially communicable, pose fish and human health constraints in integrated animal-fish farming in the Philippines (Velasquez, 1980).

About 45 species of Salmonellae have been reported in the Philippines. Tables 4 and 5 show the species recorded in or regarded as transmissible from domestic animals to man.

Lewis (1975) found that under laboratory conditions, *S. typhimurium* was taken up by the gulf killifish (*Fundulus heteroclitus*), pompano (*Trachinotus carolinus*), striped mullet (*Mugil* sp.), channel catfish (*Ictalurus punctatus*) and lake shrimp (*Penaeus setiferus*) within 2 hours of exposure. Salmonellae were recovered from the alimentary tracts of the mullet, pompano and catfish; the latter had symptoms of infection.

Fish could, therefore, be important in the epidemiology of Salmonellae. Amoebiasis (Amoebic dysentery) and balantidiasis (Balantidial dysentery) occur in areas of poor environment. Association with hogs and animal manure as fertilizer may result in higher incidence of the disease.

Fatal human cases of primary amoebic meningoencephalitis (PAM) have been reported worldwide. The causative agents are species of *Naegleria* and *Acanthamoeba* which are pathogenic free-living amoebae.

Recently, 2 intestinal samples from *Sarotherodon niloticus* (= *Tilapia nilotica*) from the BAEx (Bureau of Agricultural Extension) experimental integrated animal-fish ponds in Los Baños, Laguna were found tentatively positive for *Coccolobacilli*, *Escherichia coli* and *Neisseria polymorpha* by the Disease Diagnostic Laboratory, College of Veterinary Medicine, University of the Philippines. The samples were referred to the above agency by the author.

The above records show that knowledge of microbial diseases of fish in the Philippines is limited and fragmentary. The causative agents must be specifically identified if their relationship with the fish host is to be understood.

More studies should be done to fill up the existing gaps in our knowledge. Qualified manpower is badly needed; so are adequate facilities.

## Platyhelminthes

### Monogenea

Most Monogenea have a direct life cycle. Fig. 2 shows a diagrammatic representation of the life cycles of monogeneans. This group includes many widely distributed ectoparasitic Platyhelminthes of fishes infesting the gills and skin of fish and are frequently pathogenic to the host. The mature worms have well

Table 4. Salmonellae reported in the Philippines from farm animals and man

<i>Serotypes</i>	<i>Host/Hosts/Sources</i>	<i>References</i>
<i>S. aberdeen</i>	fowl, cattle, cow, man	Tacal & Meñez, 1968
<i>S. anatum</i>	duck, chicken, turkey cat, dog, swine, horse, carabao, cattle, man fish meal	Arambulo, 1971 Tacal & Soriano, 1970 Tacal & Meñez, 1974
<i>S. choleraesuis</i>	swine, man	Arambulo, 1971
<i>S. choleraesuis</i> var. <i>kunzendorf</i>	swine	Tacal & Meñez, 1967
<i>S. derby</i>	chicken, chicken ascarids dog, swine, houseflies	Tacal <i>et al.</i> , 1972, 1974 Arambulo, 1971
<i>S. enteritidis</i>	cattle, various animals	- ditto -
<i>S. give</i>	duck, fowl, swine, man	- ditto -
<i>S. havana</i>	chicken, man	- ditto - Tacal <i>et al.</i> , 1972 Tacal & Meñez, 1968
<i>S. javiana</i>	chicken, pigeon, swine, cattle, carabao, man	Arambulo, 1971 Tacal <i>et al.</i> , 1972
<i>S. lexington</i>	swine, man	Arambulo, 1971
<i>S. newport</i>	dog, swine, man	- ditto -
<i>S. panama</i>	fowl, dog, swine, man	- ditto -
<i>S. paratyphi</i> (types A & B)	chicken, cattle, cow man	- ditto - - ditto -
<i>S. potsdam</i>	man	Arambulo, 1971
<i>S. pullorum</i>	chicken (Table 5)	Topacio & Banci, 1965 Tacal & Soriano, 1970 Tacal <i>et al.</i> , 1970
<i>S. saint-paul</i>	duck, turkey, man	Arambulo, 1971
<i>S. seftenberg</i>	duck, dog, man	- ditto -
<i>S. singapore</i>	chicken	Tacal <i>et al.</i> , 1972 Tacal & Meñez, 1974 Tacal & Soriano, 1970
<i>S. stanley</i>	chicken, turkey, dog, pigeon, swine, cattle carabao	Tacal <i>et al.</i> , 1972 Tacal & Meñez, 1968
<i>S. virchow</i>	chicken, man	- ditto - Tacal <i>et al.</i> , 1972
<i>S. weltevreden</i>	chicken, turkey, swine, man	- ditto - Tacal & Soriano, 1970
<i>S. worthington</i>	fowl, swine, man	Arambulo, 1971

Table 5. Salmonellae reported in the Philippines from animals potentially transmissible to man

<i>Serotypes</i>	<i>Reservoir</i>
<i>S. alchua</i>	carabao
<i>S. blockley</i>	chicks, rabbit
<i>S. brunei</i>	from cockroaches and house lizard droppings
<i>S. choleraesuis</i> var <i>kunzendorf</i>	swine
<i>S. derby</i>	swine
<i>S. dublin</i>	carabao
<i>S. heidelberg</i>	swine
<i>S. hvittingfoss</i>	carabao, cattle
<i>S. java</i>	swine, carabao, cattle
<i>S. kentucky</i>	swine
<i>S. lexington</i>	swine
<i>S. pullorum</i>	chickens
<i>S. newington</i>	dog, swine
<i>S. saint-paul</i>	duck
<i>S. scerro</i>	duck
<i>S. seftenberg</i>	dog
<i>S. sieburg</i> (Group K)	dog, horse
<i>S. thompson</i>	cat
<i>S. typhimurium</i>	ducklings, chicks, dog, swine, cattle
<i>S. typhimurium</i> var. <i>copenhagen</i>	dog, swine

Arambulo, 1971; Tacal *et al.*, (1967, 1968, 1970, 1972, 1974) and Topacio *et al.* (1965).

developed posterior organs of attachment (haptor), provided with sclerotized clamps and hooks. The direct developmental cycle is completed without alternation of generations (mono=one, genea= origin). Some are viviparous (as in *Dactylogyrus*) and others are oviparous (as in *Gyrodactylus*). In viviparous worms, an embryo is formed within the mother and within that embryo is another embryo, within which a third and very often a fourth is formed, producing a high intensity of infection in infected fish. The majority of the monogenea are oviparous. Sexually mature worms deposit their eggs directly in the water. The motile ciliated larva (oncomiracidium) hatch out into the water and after a short free swimming period settle on a suitable fish host where they reach sexual maturity.

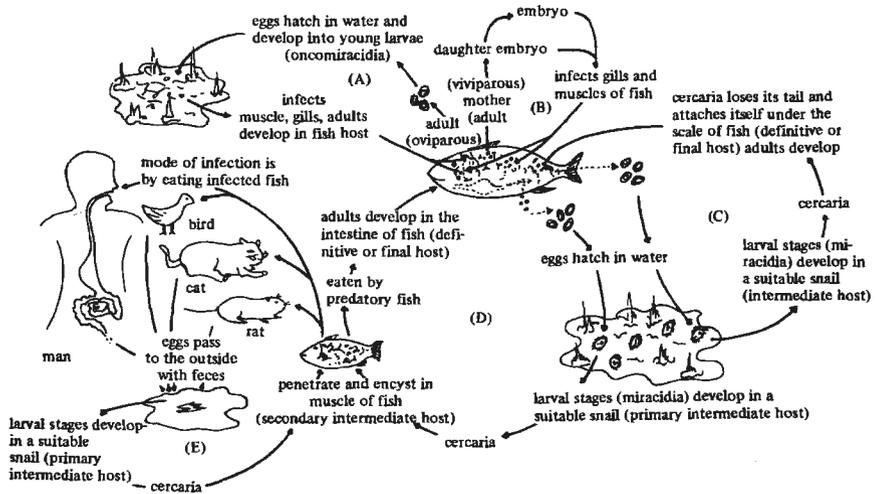


Fig. 2. Diagram of the life cycle of Monogenetic (A and B) and Digenetic (C, D and E) Trematodes.

As can be discerned in Table 6, *Dactylogyrus epizooticus* can cause economic losses to the aquaculturist in this country. As known, the source of infestation in Dactylogyrosis is either the spawners or eggs of the parasite brought into the pond which rapidly reproduce under the influence of high temperature. The intensity of infestations rises rapidly, reaching several hundreds per fish. Observations show that in about 3 days, the epizootic can kill the entire population of young fish in the pond due to severe damage of gills infested with large numbers. Fish populations should be kept at low density and infested nursery ponds must be dried, disinfected and for some time abandoned. Baths of 5% NaCl solution do not kill the worms completely. Gyrodactylosis caused by *Gyrodactylus* infection can be recognized in infested fish by its white color and lethargic condition. Mass reproduction of the parasite is enhanced by adequate temperature and high density of fish population. Mixing fish of different ages should be avoided. Being an oviparous worm, it is easily killed by dessication. *Cichlidogyrus sclerosus* which had caused some damage to the gills of *Tilapia* sp. in the experimental tanks at the CLSU (Central Luzon State University), Muñoz, Nueva Ecija is now recorded in the Philippines with the introduction of *Tilapia* spp. for aquaculture. It was originally found in Africa and the Middle East.

Now known are 6 species of Capsaloidea in one family and subfamily Velasquez (1982). They are included in the list since some of the fish host have aquaculture potential.

Table 6. Recent contributions on the Monogenea from Philippine fishes

Parasite	Location	Host Species	Locality	Effects	References
<b>DACTYLOGYRIDEA</b>					
Dactylogyridae					
<i>Dactylogyrus</i> sp.	gills	<i>Clarias batrachus</i> (hito)	Pansol, Laguna Lumbang and Candaba, Pampanga	1,000 of 5,000 fish died in 2 days severe cases	Velasquez, 1975b Velasquez, 1975b
<i>Actinocleidus</i> sp.	gills	<i>Clarias macrocephalus</i> (hito)	Candaba, Pampanga	mild	Hanririypant, 1977
<i>Cichlidogyrus sclerosus</i>	gills	<i>Tilapia mossambica</i> <i>Tilapia</i> spp.	Muñoz, Nueva Ecija	severe damage	Duncan, 1977
<b>GYRODACTYLIDEA</b>					
Gyrodactylidae					
<i>Gyrodactylus</i> sp.	skin	<i>Clarias macrocephalus</i>  <i>Clarias batrachus</i> <i>Ophicephalus striatus</i> (dalag)	Los Baños, Laguna Luzon Island	skin rupture with copious effusion  mass mortalities	Velasquez, 1975b Velasquez, 1975b Kabata, 1985
<b>CAPSALOIDEA</b>					
Capsalidae					
<i>Encotylabe caballeroi</i> Velasquez, 1977	gills	<i>Lethrinus nebulosus</i> (porgy, bakutut)	Puerto Galera, Mindoro Island		Velasquez, 1977
<i>Allometabenedeniella plataxi</i> Velasquez, 1982	gills	<i>Platax orbicularis</i> (dahon gabi)	Manila Bay, Luzon Island		Velasquez, 1982
<i>Caballeroctyla philippina</i> Velasquez, 1982	gills	<i>Platax orbicularis</i>			↓ Velasquez, 1982
<i>Neobenedenia manilae</i> Velasquez, 1982	gills	<i>Platax orbicularis</i>	Manila Bay, Luzon Island		
<i>Benedenia malaboni</i> Velasquez, 1982	gills	<i>Epinephelus undulosus</i> (lapu-lapu; grouper)	Dagat-dagatan, Malabon Rizal, Luzon Island		
* <i>Tristomella pricei</i> (Hidalgo, 1959) Price, 1960		<i>Sphyræna</i> sp.	Visayan Islands		Velasquez, 1982

\*The single specimen was given to the author by Lolita Santos Basio, Manila Medical Center. The host as indicated may have been misidentified.

It is to be noted that some Monogenea causing epizootics in this country are of economic importance but not of public health significance.

## Digenea

Aside from Tubangui's (1947) checklist on the Digenea, Fischthal and Kuntz (1964, a, b, c) from Palawan fishes, fifty-three species in 38 genera and 16 families of Trematoda of Philippine fishes by Velasquez, 1975 are now known. The list of host species and their parasites are in Table 7. It will be noted that freshwater, brackishwater and marine fishes harbor digenetic trematodes. This offers a full range of habitats of our fishes that are commercially important in our country. The zoogeographical affinities are in Table 8.

Adult trematodes usually inhabit the lumen of the intestines of fish but can be found in almost other conceivable organs. Fish serve as definitive host or as second intermediate host. The life cycles of the Trematoda may involve one or 3 hosts with several stages that are morphologically and biologically different i.e. eggs, miracidiae, sporocysts, rediae, cercariae, and metacercariae. In some instances 1 or 2 stages may not be involved. Experimental evidences show that *Transversotrema laruei*, and ectoparasitic Digenea found in many pond fishes in the Philippines, develop in redia, cercaria, is progenetic and becomes adult under the scales of the infected fish (Fig. 3) (Velasquez, 1961). The "whirling" syndrome in *Tilapia* spp. in Pililia, Rizal fishponds is associated with this parasite.

Pathological changes in the skin and subcutaneous tissues are caused by the metacercariae of trematodes. Fig. 4 shows *Neodiplostomum* sp. metacercaria infected scale of *Ophicephalus striatus*. Holes produced indicate the presence of a lytic substance. Massive invasion of penetrating cercaria can cause blindness or death to fish especially small ones. Serious mechanical injuries may result. The metacercaria of *Clinostomum philippinensis* Velasquez, 1959 encyst in the eyes of *Ophicephalus striatus*. However minimal is the effect, every parasite exerts some degree of influence which may be regarded as pathogenic when extensive changes in the individual organs occur.

Transfer of fish to new environment or acclimatization in ponds requires a full understanding of the complete life cycle of causative agents of disease. Prophylactic measures for the successful prevention and control of parasites can only be attained with such knowledge. Fig. 2 shows a diagrammatic representation of a generalized pattern of the life cycles of the Digenea and Monogenea.

Nine life cycles of trematodes have been demonstrated experimentally, 4 of which are from fishes (Velasquez, 1961, 1973a, b, 1975); two are of public health significance, that of *Transversotrema laruei* Velasquez, 1958 is not only significant zoogeographically and phylogenetically but also in fish health management. The life cycle of *Orientocreadium batrachoides* in *Clarias macrocephalus* has been studied (Sirikantayakul, 1985) (Fig. 5).

The distribution of parasites is dependent on (1) the presence of suitable hosts and (2) habits and environmental conditions that make transfer from host to

Table 7. Digenetic trematodes of Philippine fishes

<i>Host-species and their parasites</i>		
<i>Host-species</i>	<i>Common name (Tagalog)</i>	<i>Parasites</i>
Anguillidae		
*** <i>Anguilla mauritiana</i> Bennett	“igat”	<i>Galactosomum anguillarum</i> (Tubangui, 1933) Tubangui & Africa, 1938
Ambassidae		
*** <i>Ambassis buruensis</i> Bleeker	“langaray”	<i>Prosorhynchus paracrucibulus</i> Velasquez, 1959
Ariidae		
** <i>Arius thalassinus</i> (Rüppell)	“kanduling-dagat”	<i>Monodhelmins philippinensis</i> Velasquez, 1961 <i>Pelorohelmins palawanensis</i> Fischthal & Kuntz, 1964
Carangidae		
** <i>Caranx affinis</i> Rüppell	“talakitok”	<i>Lecithocladium angustiovum</i> Yamaguti, 1953
** <i>Caranx</i> sp.	ditto	<i>Alcicornis cirrudiscoides</i> Velasquez, 1959 <i>Buscephalus varicus</i> Manter, 1940 <i>Prosogonotrema carangis</i> Velasquez, 1961
*** <i>Megalaspis cordyla</i> (Linn.)	“ oriles” “uriles”	<i>Bucephalus fragilis</i> Velasquez, 1959 <i>Lecithocladium megalaspis</i> Yamaguti, 1953
*** <i>Scomberoides lysan</i> (Forsk.)	“dorado” “lapis”	<i>Erilepturus lemeriensis</i> (Tubangui & Masiluñgan, 1935)
*** <i>Scomberoides</i> sp.	“dorado”	<i>Erilepturus lemeriensis</i> (Tubangui & Masiluñgan, 1935)
*** <i>Seriola nigrofasciata</i> (Rüppell)	“lapis”	<i>Bucephalus paraheterotentaculatus</i> Velasquez, 1959
Chaetodontidae		
*** <i>Parachaetodon ocellatus</i>	“paru-paru”	<i>Hurleytrematoides malabonensis</i> Velasquez, 1961
Clupeidae		
*** <i>Harengula dispilonotus</i>	“tambang bato”	<i>Aphanurus stossichi</i> (Monticelli, 1891) Looss, 1907
Dorosomatidae		
*** <i>Anodontostoma</i> (= <i>Dorosoma</i> ) <i>chacunda</i> Hamilton	“kabasi”	<i>Gyliauchen papillatus</i> (Goto & Matsudaira) 1918

Table 7 continued

<i>Host-species and their parasites</i>		
<i>Host-species</i>	<i>Common name (Tagalog)</i>	<i>Parasites</i>
Engraulidae		
*** <i>Stolephorus commersoni</i> (Lacepede)	"dilis"	<i>Hexangium sigani</i> Goto & Ozaki, 1929
Gobiidae		
* <i>Glossogobius giurus</i> Buchanan-Hamilton	"biyang puti"	<i>Azygia pristipomai</i> Tubangui, 1928 (neotype) <i>Erilepturus lemeriensis</i> (Tubangui & Masiluñgan, 1935) Manter, 1947 <i>Neochasmus microvatus</i> (Tubangui, 1928) Tubangui & Masiluñgan, 1944
Holocentridae		
* <i>Holocentrus sammara</i> (Forsk.)	"baga-baga"	<i>Hexangium sigani</i> Goto & Ozaki, 1929
Labridae		
*** <i>Cheilinus diagrammus</i> (Lacepede)	"mulmul"	<i>Transversotrema haasi</i> Witenberg, 1944
** <i>Cheilio inermis</i> (Forsk.)		<i>Brachadena cheilionis</i> Fischthal & Kuntz, 1964
Leiognathidae		
** <i>Gazza minuta</i> Bloch	"motambo"	<i>Peiorohelminis palawanensis</i> Fischthal & Kuntz, 1964 <i>Rhipidocotyle eggletoni</i> Velasquez, 1959
** <i>Leiognathus</i> sp.	"sapsap"	<i>Bucephalus leiognathi</i> Velasquez, 1959
Lutianidae		
** <i>Caesio erythrogaster</i> Cuvier & Valenciennes		<i>Hexangium sigani</i> Goto & Ozaki, 1929
*** <i>Lutianus fulviflamma</i> (Forsk.)	"bitilla"	<i>Paracryptogonimus manilensis</i> Velasquez, 1961 <i>Paracryptogonimus orientalis</i> Fischthal & Kuntz, 1964
** <i>Lutianus</i> sp.	"maya-maya" "bambangin"	<i>Paracryptogonimus acanthostomus</i> Yamaguti, 1934 <i>Paracryptogonimus apharei</i> (Yamaguti, 1942) Velasquez, 1961 <i>Pseudallacanthochasmus grandispinus</i> Velasquez, 1961

Table 7 continued

<i>Host-species and their parasites</i>		
<i>Host-species</i>	<i>Common name (Tagalog)</i>	<i>Parasites</i>
*** <i>Lutianus vitta</i> (Quoy & Gaimard)	"maya-maya"	<i>Paracryptogonimus manilensis</i> Velasquez, 1961
Nemipteridae		
** <i>Gymnocranius griseus</i> (Schlegel)	"katumbal"	<i>Propycnadenoides philippinensis</i> Fischthal & Kuntz, 1964
Pomadasyidae		
*** <i>Pristipoma hasta</i> (Cuvier & Valenciennes) (= <i>Pomadasy hasta</i> ) (Bloch)	"ikuran"	<i>Azygia pristipomai</i> Tubangui, 1928 (neotype)
*** <i>Pomadasy hasta</i> (Bloch)		<i>Aephniidiogenes barbarus</i> Nicoll, 1915
Psettodidae		
*** <i>Psettodes erumei</i> (Bloch & Schneider)	"dapa"	<i>Prosorhynchus longus</i> Velasquez, 1959 <i>Rhipidocotyle laruei</i> Velasquez, 1959
Scaridae		
*** <i>Scarus lunula</i> (Snyder)	"mulmol"	<i>Transversotrema haasi</i> Witenberg, 1944
Scatophagidae		
*** <i>Scatophagus argus</i> (Linn.)	"kitang"	<i>Warcotrema piscicola</i> Srivastava, 1939
Scombridae		
** <i>Euthynnus yaito</i> (Kishinouye)	"tulingan" "kucharita"	<i>Lecithochirium magnaporum</i> Manter, 1940
*** <i>Rastrelliger chrysozonus</i> (Ruppell)	"alumahan"	<i>Lecithocladium angustiovum</i> Yamaguti, 1953
Serranidae		
** <i>Epinephelus bleekeri</i> (Vaillant & Bocour)	"lapu-lapu"	<i>Prosorhynchus mcintoshi</i> (Velasquez, 1959) Yamaguti, 1971
*** <i>Lates calcarifer</i> Bloch	"apahap"	<i>Lecithochirium neopacificum</i> Velasquez, 1962 <i>Pseudometadena celebesensis</i> Yamaguti, 1952 <i>Prosorhynchus luzonicus</i> Velasquez, 1959 <i>Transversotrema laruei</i> Velasquez, 1958
Sillaginidae		
*** <i>Sillago sihama</i> (Forsk.)	"asohos"	<i>Rhipidocotyle eggletoni</i> Velasquez, 1959

Table 7 continued

<i>Host-species and their parasites</i>		
<i>Host-species</i>	<i>Common name (Tagalog)</i>	<i>Parasites</i>
Sparidae		
*** <i>Sparus berda</i> (Forsk.)	"bakokong moro"	<i>Prosohynchus crucibulus</i> (Rudolphi, 1819) Odhner, 1905
Sphyraenidae		
*** <i>Sphyraena langsar</i> Bleeker	"barracuda"	<i>Bucephaloides philippinorum</i> Velasquez, 1959
*** <i>Sphyraena</i> sp.	"tursilyo"	<i>Didymozoon bravohollisae</i> Velasquez, 1970
Stromateidae		
** <i>Apolectus niger</i> (Bloch)	"pampano"	<i>Lecithocladium apolecti</i> Velasquez, 1962
Teuthidae (Siganidae)		
*** <i>Amphacanthus javus</i> Linn.	"samaral"	<i>Hexangium sigani</i> Goto & Ozaki, 1929
*** <i>Siganus fuscescens</i> Seale & Bean	ditto	<i>Gyliauchen papillatus</i> (Goto & Matsudaira, 1918) Goto, 1919 <i>Hexangium sigani</i> Goto & Ozaki, 1929
*** <i>Siganus guttatus</i> Bloch	ditto	ditto
*** <i>Siganus oramin</i> (Bloch & Schneider)	ditto	ditto
*** <i>Siganus</i> sp.	ditto	ditto
*** <i>Siganus striolatus</i> Seale	ditto	<i>Hemirurus sigani</i> Fischthal & Kuntz, 1964
*** <i>Teuthis concatenata</i> (Cuvier & Valenciennes)	ditto	ditto
*** <i>Teuthis rostrata</i> (Cuvier & Valenciennes)	ditto	ditto
Theraphonidae		
*** <i>Therapon argenteus</i> (Cuvier & Valenciennes)	"ayungin"	<i>Azygia pristipomai</i>  Tubangi, 1928 (neotype)
*** <i>Therapon jarbua</i> (Cuvier & Valenciennes)	"bagaong"	ditto

Table 7 continued

<i>Host-species and their parasites</i>		
<i>Host-species</i>	<i>Common name (Tagalog)</i>	<i>Parasites</i>
Trichiuridae ** <i>Trichiurus haumela</i>	"pinka"	<i>Plerurus longicaudatus</i> (Yamaguti, 1953) Manter et Pritchard, 1960
* - freshwater		
** - marine		
*** - marine entering rivers (estuarine)		

host possible. Predators, intermediate hosts, food habits are biotic factors in the transmission of parasites.

Those capable of infecting many different kinds of hosts are polyxenous and hence have a wider distribution and those that are host specific are monoxenous.

Fish as carriers of human helminthic infections are listed in Table 9. Most infections of piscivorous mammals, birds and man occur upon ingestion of infected fish flesh, either raw or not well cooked. Cardiac and visceral complications in humans are known in the Philippines (Africa *et al.*, 1940). The helminthic parasites of domestic animals for which cases of human infection have been recorded in the Philippines are presented in Table 10.

Contamination of water with feces of reservoir animals continue to be a public health hazard. Available evidences indicate that some parasites of farm animals and freshwater fish may pose questions for public health. The farmer's responsibility in maintaining sanitation and hygiene cannot be overlooked and strong government regulations regarding health measures are required. Research in the profitable recycling of our wastes should continue.

The life cycles of *Haplorchis taichui* and *Procerovum calderoni* have been experimentally demonstrated by Velasquez, 1973a, b. *Melania juncea* Lea, the snail intermediate host of *Haplorchis taichui*, is commonly found in rice paddies. *Thiara riquetti* a brackishwater snail, the intermediate host of *Procerovum calderoni* and *Transverstroma laruei*, abound in banḡus (*Chanos chanos*) and *Tilapia* fishponds. *Carneophallus brevicacea* (Africa and Garcia, 1935) Velasquez, 1975 was originally considered the causative agent of heterophyiasis. The developmental pattern is that of a microphallid, although the symptoms are those of a heterophyid (Velasquez, 1975). It is proposed therefore, that the disease be called micro-

phalliasis. Continuous eating of raw naturally-infected intermediate host, *Macrobrachium* sp. (Fig. 6), produces lethal or sublethal infections of the heart, spinal cord and vital organs. In certain areas of the Philippines, the shrimp is eaten raw with table condiments such as vinegar, tomatoes and salt.



Fig. 3. *Transversotrema laruei* Velasquez, 1958.

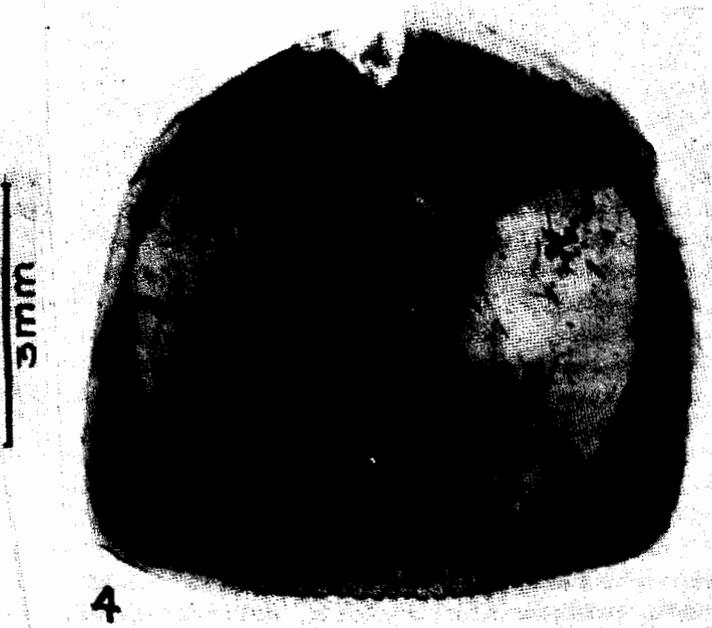


Fig.4. Scale of *Ophicephalus striatus* infected with *Neodiplostomum* sp. metacercaria. Note hole produced.

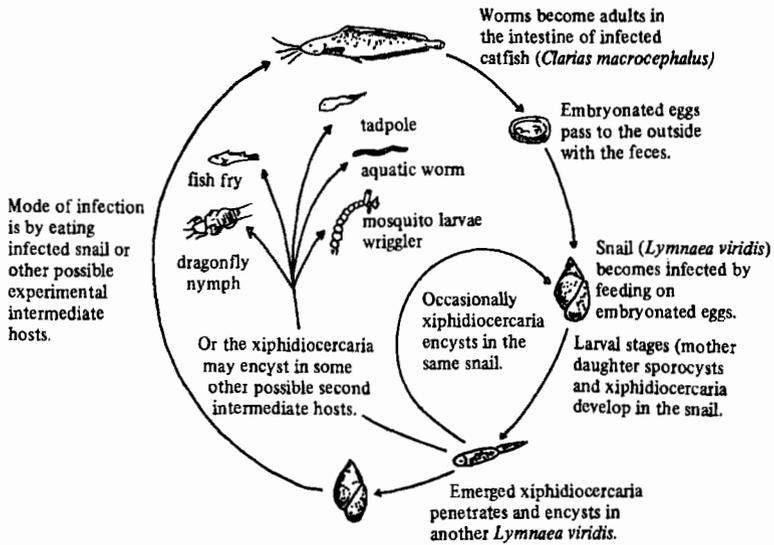


Fig. 5. Life cycle of *Orientocreadium batrachoides* Tubangui, 1931 as experimentally demonstrated in *Clarias macrocephalus* Gunther, 1864. (from Sirikantayakul, 1985).



Fig. 6. *Macrobrachium* sp. intermediate host of *Carneophallus brevicaca* (Africa and Garcia, 1935) Velasquez, 1975.

Table 8. List of some previously described parasitic helminths of fishes with new host and/or locality records

<i>Parasite</i>	<i>Type Host</i>	<i>Type Locality</i>	<i>New Host***</i>		<i>New Locality</i>
			<i>Scientific Name</i>	<i>Common</i>	
<b>TREMATODES:</b>					
Family Bucephalidae					
Poche, 1907					
1. <i>Prosorhynchus crucibulus</i> (Rudolphi, 1819) Odhner, 1905	<i>Muraena conger</i>	Mediterranean	<i>Sparus berda</i> (Forskål)	"bakokong moro"	Manila Bay, Parañaque, Rizal
Family Azygiidae					
Odhner, 1911					
2. <i>Azygia pristipomai</i> Tubangui, 1928*	<i>Pristipoma hasta</i> Bloch	Laguna de Bay, Los Baños, Laguna	<i>Therapon argenteus</i> (Cuvier)	"ayungin"	Sta. Rosa, Laguna, Tanay, Rizal
Family Waretrematidae					
Srivastava, 1939					
3. <i>Waretrema piscicola</i>	<i>Mugil waigensis</i>	Karachi, Arabian Sea	<i>Scatophagus argus</i> (Linn.)	"kitang"	Obando, Bulacan
Family Angiodictyidae					
Looss, 1902					
4. <i>Hexangium sigani</i>	<i>Siganus fuscescens</i> syn. <i>Teuthis fuscescens</i> (Moultuyn)	Misaki, Takamatsu, Japan	<i>Teuthis rostrata</i> (Cuvier & Valenciennes)	"samaral"	Dagat-dagatan, Malabon Rizal

Table 8 continued

Parasite	Type Host	Type Locality	New Host ***		New Locality
			Scientific Name	Common	
Family Cryptogonimidae					
Ciurea, 1933					
5. <i>Paracryptogonimus apharei</i> (Yamaguti, 1957) new comb. Vel. 1961	<i>Aphareus furcatus</i> (Lacepede)	Naha, Okinawa,	<i>Lutianus</i> sp.		Malabon (Open sea), Rizal
6. <i>Neochasmus microvatus</i> (Tubangui 1928) Tubangui et Masiluñgan, 1944** syn. <i>Metadena microvata</i> Tubangui, 1928 <i>Metadena ovata</i> Tubangui & Francisco, 1930	<i>Glossogobius giurus</i> (Buchanan & Hamilton)	Laguna de Bay, Los Baños, Laguna	<i>Glossogobius giurus</i>	"biang puti"	Pasig, Alabang, Cardona, Binañonan, Rizal; Sukat, Cavite; Sta. Rosa, Laguna
7. <i>Paracryptogonimus acanthostomus</i> Yamaguti, 1954	<i>Lutianus vitta</i> (Quoy & Gaimard)	Nakayama, Prefecture, Japan	<i>Lutianus</i> sp.	"bambañgin"	Malabon, Rizal
8. <i>Pseudometadena celebesensis</i>	<i>Lates calcarifer</i> (Bloch)	Macassar, Celebes	<i>Lates calcarifer</i> (Bloch)	"apahap"	Malabon, Rizal
Family Hemiuridae					
Luehe, 1901					
9. <i>Erilepturus lemeriensis</i> (Tubangui & Masiluñgan, 1935) Manter, 1947*	<i>Scomberoides</i> sp.	Balayan, Lemery, Batangas	<i>Scomberoides lysan</i> (Forskål)	"Dorado"	Navotas & Malabon, Rizal

Table 8 continued

Parasite	Type Host	Type Locality	New Host***		New Locality
			Scientific Name	Common	
10. <i>Lecithocladium angustiovum</i> Yamaguti, 1953	<i>Scomber kanagurta</i> (Cuvier)	Macassar, Celebes	<i>Rastrelliger chrysozonus</i> (Rupell) syn. <i>Scomber microlepidotus</i>	"hasa-hasa"	Malabon, Rizal Visayan Islands (bought from Quiapo market)
11. <i>Lecithocladium megalaspis</i> Yamaguti, 1953	<i>Megalaspis</i> sp.	Macassar, Celebes	<i>Megalaspis cordyla</i> (Linn.)	"tulay"	Malabon, Rizal
NEMATODES:					
Family Camallanidae					
Railliet et Henry, 1915					
12. <i>Camallanus ophicephali</i> Pearse, 1933	<i>Ophicephalus striatus</i> (Bloch)	Bangkok, Thailand	<i>Ophicephalus striatus</i>	"dalag"	Laguna de Bay, Los Baños, Laguna
13. <i>Camallanus carangis</i> Olsen, 1954	<i>Caranx</i> sp.	Fiji Is.	<i>Caranx</i> sp.	"talakitok"	Malabon, Rizal
14. <i>Zeylanema anabantis</i> (Pearse) Yeh, 1960	<i>Anabas testudineus</i> (Bloch)	Bangkok, Thailand, Malaysia	<i>Anabas testudineus</i> Bloch	"martiniko"	Laguna de Bay, Los Baños Laguna
15. <i>Spirocamallanus spiralis</i> (Baylis, 1923) Olsen, 1952	<i>Heterobranchus anguillaris</i>	Egypt	<i>Nemipterus</i> , sp. <i>Platycephalus indicus</i> (Linn.)	"bjsugo" "sunog"	Malabon & Navotas, Rizal

Table 8 continued

<i>Parasite</i>	<i>Type Host</i>	<i>Type Locality</i>	<i>Scientific Name</i>	<i>New Host *** Common</i>	<i>New Locality</i>
			<i>Teuthis</i> sp.	"samaral"	
			<i>Sparus berda</i> (Forskäl)	"bakoko"	
			<i>Tylosurus</i> sp.	"batalay"	Hagonoy, Bulacan

Legend:      \*Neotype      \*\*Topotype      \*\*\*Except numbers 6 and 12.

Table 9. List of Philippine fishes harboring larvae of parasitic helminths transmissible to man

Name of Fish	Tagalog Name	Habitat	Name of Worm	
			Trematodes	Nematodes
<i>Anabas testudineus</i>	"martiniko"	freshwater	<i>Procerovum calderoni</i> * <i>Stellantchasmus amplicacalis</i> * <i>Centrocestus caninus</i>	<i>Gnathostoma spinigerum</i> *
<i>Arius manilensis</i>	"kanduli"	freshwater	<i>Haplorchis yokogawai</i> *	
<i>Clarias batrachus</i> L.	"hito"	freshwater	<i>Haplorchis yokogawai</i> *	
<i>Glossogobius giurus</i>	"bia" or "biang puti"	freshwater	<i>Procerovum calderoni</i> * <i>Haplorchis pumilio</i> <i>Centrocestus caninus</i>	<i>Gnathostoma spinigerum</i> *
<i>Ophicephalus striatus</i>	"dalag" or "bulig"	freshwater	<i>Procerovum calderoni</i> <i>Haplorchis yokogawai</i> * <i>Haplorchis pumilio</i>	

Table 9 continued

Name of Fish	Tagalog Name	Habitat	Name of Worm	
			Trematodes	Nematodes
<i>Ophicephalus striatus</i>			<i>Haplorchis taichui*</i>	
			<i>Clinostomum philippinensis</i>	<i>Gnathostoma spinigerum*</i>
<i>Puntius binotatus</i> Cuvier & Valenciennes	“bitño” (Maranao)	freshwater	<i>Haplorchis pumilio</i>	
			<i>Haplorchis taichui*</i>	
<i>Puntius palata</i> Fowler Syn. of <i>Spratellicypris palata</i> Herre	“bitño” (Maranao)	freshwater	<i>Haplorchis taichui*</i>	
<i>Therapon plumbeus</i>	“ayunġin”	freshwater	<i>Haplorchis pumilio</i>	
			<i>Centrocestus caninus</i>	
<i>Therapon argenteus</i> Cuvier & Valenciennes	“ayunġin”	freshwater to saltwater		<i>Gnathostoma spinigerum*</i>
<i>Ambassis buruensis</i> Bleeker	“lañgaray”	freshwater to brackish to marine	<i>Stictodora guerreroi</i>	
			<i>Stictodora manilensis</i>	
			<i>Procerovum calderoni*</i>	
			<i>Haplorchis pumilio</i>	
			<i>Heterophyopsis expectans</i>	

Table 9 continued

Name of Fish	Tagalog Name	Habitat	Name of Worm	
			Trematodes	Nematodes
<i>Atherina balabacensis</i> Seale Syn. of <i>Hepsetia</i> <i>balabacensis</i> (Seale)	"guno"	marine to brackish occasionally to salt water	<i>Stictodora</i> <i>guerreroi</i> <i>Stictodora</i> <i>manilensis</i> <i>Procerovum</i> <i>calderoni</i> * <i>Heterophyopsis</i> <i>expectans</i>	
<i>Caranx armatus</i> (Forsk.)	"talakitok"	marine to brackish		<i>Anisakis</i> sp.**
<i>Chanos chanos</i> (Forsk.)	"bañgus"	marine to brackish to mouth of rivers	<i>Procerovum</i> <i>calderoni</i> *	
<i>Decapterus macrosoma</i> Bleeker	"galunggong"	marine		<i>Anisakis</i> sp.**
<i>Eleutheronema</i> <i>tetradactyla</i> Shaw	"mamale"	marine occasionally to brackish	<i>Procerovum</i> <i>calderoni</i> *	
<i>Euthynnus yaito</i> Kishinouye	"tuliñgan"	marine		<i>Anisakis</i> sp.**
<i>Gerres kapas</i> Bleeker	"malakapas"	marine to brackish to freshwater	<i>Haplorchis</i> <i>yokogawai</i> * <i>Heterophyopsis</i> <i>expectans</i>	
<i>Gerres filamentosus</i> Cuvier	"malakapas"	marine to brackish to freshwater	<i>Stictodora</i> <i>manilensis</i>	

Table 9 continued

Name of Fish	Tagalog Name	Habitat	Name of Worm	
			Trematodes	Nematodes
<i>Gerres filamentosus</i> Cuvier			<i>Heterophyopsis expectans</i> <i>Procerovum calderoni*</i> <i>Haplorchis pumilio</i>	
<i>Hemiramphus georgii</i> Cuvier & Valenciennes	"kansusuit" or "buguing"	marine to brackish occasionally freshwater	<i>Stictodora guerreroi</i> <i>Procerovum calderoni*</i> <i>Haplorochis yokogawai*</i>	
<i>Lutianus vitta</i> Quoy & Gaimard	"maya-maya"	marine		<i>Anisakis</i> sp. **
<i>Mene maculata</i> Bloch & Schneider	"bambangin"	marine		<i>Anisakis</i> sp. **
<i>Mugil dussumieri</i> Cuvier & Valenciennes	"talilong" or "banak"	salt water to brackish to freshwater	<i>Stictodora guerreroi</i> <i>Stictodora manilensis</i> <i>Procerovum calderoni*</i> <i>Haplorchis yokogawai*</i> <i>Stellani chasmus amplicaecalis</i> <i>Heterophyopsis expectans</i>	

Table 9 continued

Name of Fish	Tagalog Name	Habitat	Name of Worm	
			Trematodes	Nematodes
<i>Mugil dussumiere</i> Cuvier Valenciennes			<i>Pygidiopsis marivillai</i> <i>Pygidiopsis genata</i>	
<i>Pelates quadrilineatus</i> (Bloch)	"babansi"	marine to brackish occasionally to fresh	<i>Stictodora manilensis</i> <i>Procerovum calderoni*</i> <i>Heterophyopsis expectans</i>	
<i>Platax orbicularis</i> (Forskāl)	"dahong-gabi"	marine		<i>Anisakis</i> sp.**
<i>Scatophagus argus</i> L.	"kitang"	marine to brackish to fresh	<i>Procerovum calderoni*</i>	
<i>Selaroides leptolepis</i> Cuvier	"salay-salay"	marine		<i>Anisakis</i> sp.**
<i>Sparus berda</i> Forskāl	"bakoko"	marine		<i>Anisakis</i> sp.**
<i>Stolephorus</i> sp.	"dilis"	marine		<i>Anisakis</i> sp.**
<i>Teuthis javus</i> L.	"samaral"	marine occasionally to brackish	<i>Procerovum calderoni*</i> <i>Haplorchis pumilio</i> <i>Heterophyopsis expectans</i>	
<i>Therapon puta</i>	"boloan"	marine		<i>Anisakis</i> sp.**

Legend: \*Encysted in tissues; worms recovered from the intestines of man in the Philippines.

\*\*Worms recovered from fatal human cases in Holland.

Table 10. Helminths of lower animals reported in the Philippines with records of human infection<sup>1</sup>

Parasite	Natural Host/Hosts in Lower Animals	Intermediate Host/ Hosts	Common Name (Tagalog)	Organ (Human)	Locality (Human case/ cases)
<b>TREMATODA (Digenea)</b>					
Family Echinostomatidae					
Looss, 1902					
<i>Echinostoma ilocanum</i>	rat	1° <i>Anisus convexisculus</i> (snail) 2° <i>Pila luzonica</i> (snail)	suso  kuhol	intestines	Northwestern Luzon, Manila, Zambales, Leyte, Mindoro, Mindanao
Family Fasciolidae					
<i>Fasciola hepatica</i>	cattle, carabao	1° <i>Lymnaea</i> spp. (snail)		liver, bile duct	Albay, Bicol provinces
<i>Fasciola gigantica</i>	cattle, carabao				
Family Heterophyidae					
Odhner, 1914					
<i>Stellantchasmus amplicaealis</i> (Katsurada, 1931) syn. of <i>Diorchitrema pseudochranta</i> (Witenberg, 1929) syn. of <i>S. falcatus</i> Onji et Nishio, 1915	dog, cat, mouse (experimental), bird	1° 2° fish <i>Anabas testudineus</i>	liwalo	intestines	Manila, Luzon
<i>Procerovum calderoni</i> Africa et Garcia, 1935 (Price, 1940)	dog, cat, duckling	1° <i>Thiara riquetti</i> snail***a		intestines	Biñan, Laguna, Luzon

Table 10. continued

Parasite	Natural Host/Hosts in Lower Animals	Intermediate Host/hosts	Common Name (Tagalog)	Organ (Human)	Locality (Human case/cases)
		2° fish			
		<i>Ophicephalus striatus</i>	dalag		
		<i>Glossogobius giurus</i>	bia		
		<i>Gerris filamentosus</i>	kapas		
		<i>Creisson validus</i>	bia		
		<i>Amphacanthus javus</i>	samaral		
		<i>Pelates quadrilineatus</i>	babansi		
		<i>Eleutheronema tetradactyla</i>	mamali		
		<i>Mugil</i> sp.	banak		
		<i>Hemiramphus georgii</i>	kansusuit		
		<i>Ambassis buruensis</i>	langaray		
		<i>Mugil dussumieri</i> *	talilong		
		<i>Chanos chanos</i>	bañgus		
		<i>Butis amboinensis</i>	biang sunog		
		<i>Platycephalus indicus</i>	sunog		
		<i>Mollienesia latipinna</i> *	bubuntis		
<i>Haplorchis taichui</i> (Nishigori, 1924) Chen, 1936	cat, dog, cattle, egret, chick (experimental)	1° <i>Melania juncea</i> ** <sup>b</sup> Lea		intestines	Manila, Luzon, Leyte
		2° fish			
		<i>Ophicephalus striatus</i>	dalag		
		<i>Puntius binotatus</i>	cyprinid		
		<i>Puntius palata</i>			
<i>Haplorchis yokogawai</i> * Katsusata, 1932 (Chen, 1936)	cat, dog, cattle, egret, monkey (experimental)	1° ? 2° fish		intestines	Manila, Luzon
		<i>Ophicephalus striatus</i>	dalag		
		<i>Gerres</i> sp.	kapas		
		<i>Amphacanthus javus</i>	samaral		
		<i>Ambassis buruensis</i>	langaray		
		<i>Hemiramphus georgii</i>	kansusuit		
		<i>Mugil</i> sp.	banak		

Table 10. continued

<i>Parasite</i>	<i>Natural Host/Hosts in Lower Animals</i>	<i>Intermediate Host/hosts</i>	<i>Common Name (Tagalog)</i>	<i>Organ (Human)</i>	<i>Locality (Human case/cases)</i>
<i>Carneophallus brevicæca</i> (Africa et Garcia, 1935) Velasquez, 1975 ***	wild bird	1° ? 2° shrimp *** fish (paratenic) <i>Glossogobius giurus</i>	bia	intestines	Manila
Family Opisthorchiidae Luehe, 1901					
<i>Clonorchis sinensis</i> (Cobbold, 1875)  Looss, 1907	dog, cat	1° ? (not found in the Philippines) 2° (not found in the Philippines)		liver (Chinese patient)	Manila, Los Baños, Laguna, Luzon
Family Troglotrematidae Odhner, 1914					
<i>Paragonimus westermani</i> Kerbert, 1878	cat, rat (experimental)	1° <i>Brotia asperata</i> 2° <i>Parathelphusa</i> ( <i>Barythelphusa</i> ) <i>mistio</i>	tabaguang (Bicol) crab	lung	Albay (Guinobatan), Camarines Sur (Naga), Sorsogon (Gubat), Luzon, Samar, Leyte, Mindanao
Family Philophthalmidae Travassos, 1921					
<i>Philophthalmus</i> sp. ****	conjunctival sac of bird, chicken	snail ?		conjunctival sac	Ilocos ****
Family Schistosomatidae Looss, 1899					
<i>Schistosoma japonicum</i>	cat, dog, pig	<i>Oncomelania quadrasi</i>		blood vessels,	Mindoro, Leyte, Samar,

Table 10. continued

<i>Parasite</i>	<i>Natural Host/Hosts in Lower Animals</i>	<i>Intermediate Host/hosts</i>	<i>Common Name (Tagalog)</i>	<i>Organ (Human)</i>	<i>Locality (Human case/cases)</i>
<i>Schistosoma Japonicum</i>	goat, cattle, horse, wild rats, experimentally in guinea pig, white rat mouse, monkey	snail		portal and mesenteric veins	Bicol provinces, Mindanao
NEMATODA					
Family Ascaridae					
Baird, 1953					
<i>Ascaris lumbricoides</i>	pig, man	none		intestines	widely distributed
Linn., 1758					
Family Gnathostomidae					
Blanchard, 1895					
<i>Gnathostoma spinigerum</i>	dog, cat, civet	1° copepods <i>Cyclops (Eucyclops) serrulatus</i> <i>C. (Microcyclops) bicolor</i> 2° fish <i>Ophicephalus striatus</i> <i>Anabas testudineus</i> <i>Glossogobius giurus</i>		sub-cutaneous	Manila, Luzon
			dalag liwalo bia		

<sup>1</sup>With aquatic intermediate hosts.

Ref. Tubangui (1947) except with asterisk.

\*Velasquez (1973)

\*\*Velasquez (1973a, b)

\*\*\*Velasquez (1975)

\*\*\*\*Velasquez (1978)

Galvez (1979) found that cysts of *Procerovum calderoni* "in situ" at 29.5°C in 5 to 15% saline solution died after 76 to 16 hours. Corresponding survivals in soy sauce and vinegar were 7 to 20 hours, respectively. Cysts in fish refrigerated at 8.5°C and then marinated in 5 to 15% salt solution survived for 70 to 220 hours. Corresponding survivals in soy sauce and vinegar were 30 and 240 hours, respectively. Freezing of infected fish for 120 hours, sun-drying for 9.5 hours and smoking for 1 hour killed all cysts. Frying or boiling killed all cysts in 10 to 15 minutes.

Cases of pharyngo-laryngitis or "halzoun" in man in the Near East have been attributed by Witenberg of Israel (1944) to the ingestion of raw or insufficiently cooked *Clinostomum* infected fish. In the Philippines, several species of clinostomids have been found in loose connective tissues, cysts in the eyes, opercular, pericardial cavities and muscles of the different parts of the body of some of our freshwater fishes such as *Ophicephalus striatus* (mudfish), *Clarias macrocephalus*, climbing perch (*Anabas testudineus*) and the carp (*Cyprinus carpio*). Fig 7. These worms mature in the mouth of fish-eating birds. Velasquez (1959) experimentally demonstrated (Fig. 8) that *Clinostomum philippinensis* Velasquez, 1959 metacercariae from *Ophicephalus striatus* can develop to maturity in the black crowned night heron, *Nycticorax nycticorax nycticorax* (Linn.), the rufous night heron *Nycticorax caledonicus manillensis* and the Least bittern, *Ixobrychus cinnamomeus*. Heavy infections by these worms slow down fish hosts and may become easy prey of other animals which serve as the next link in the food chain. Infected birds suffer from ulcerations of the buccal cavity. (Fig. 5a)

### Cestoda

The cestodes of fishes predominantly belong to the Order Pseudophyllidea and Tetracyphillidea. There are one or two intermediate hosts. The life cycles of cestodes have a more or less uniform pattern differing only in details. Fig. 9 shows the generalized life cycle of some Pseudophyllidean tapeworms. The entire life cycle is determined by the food relationships of the host animals. Tapeworm larvae do not actively penetrate the host.

The cestodes of marine fishes although found numerous, are mostly larval stages in our teleost fishes, adults being in clasmobranchs. They are not included in this paper.

*Bothriocephalus travassosi* Tubangui, 1938 from the intestine of a fish, *Anguilla mauritiana* from Palo, Leyte is the only fish cestode included in Tubangui's (1947) checklist. Hanviriyapant (1977) first found *Gangesia* in *Clarias macrocephalus* in this country. Velasquez (1978) found 2 genera and species of *Lytocestus* of the family Lytocestidae from *Clarias batrachus*. The alimentary tract of the infected fish with *Lytocestus* adult tapeworms exhibited petechiae in the sites of infection. The representatives of the genus have been earlier known in catfish in the Ethiopian and Oriental regions. Two species of Bothriocephalidae have been found recently (Velasquez, 1978). *Bothriocephalus achelognathi* from the intestine of

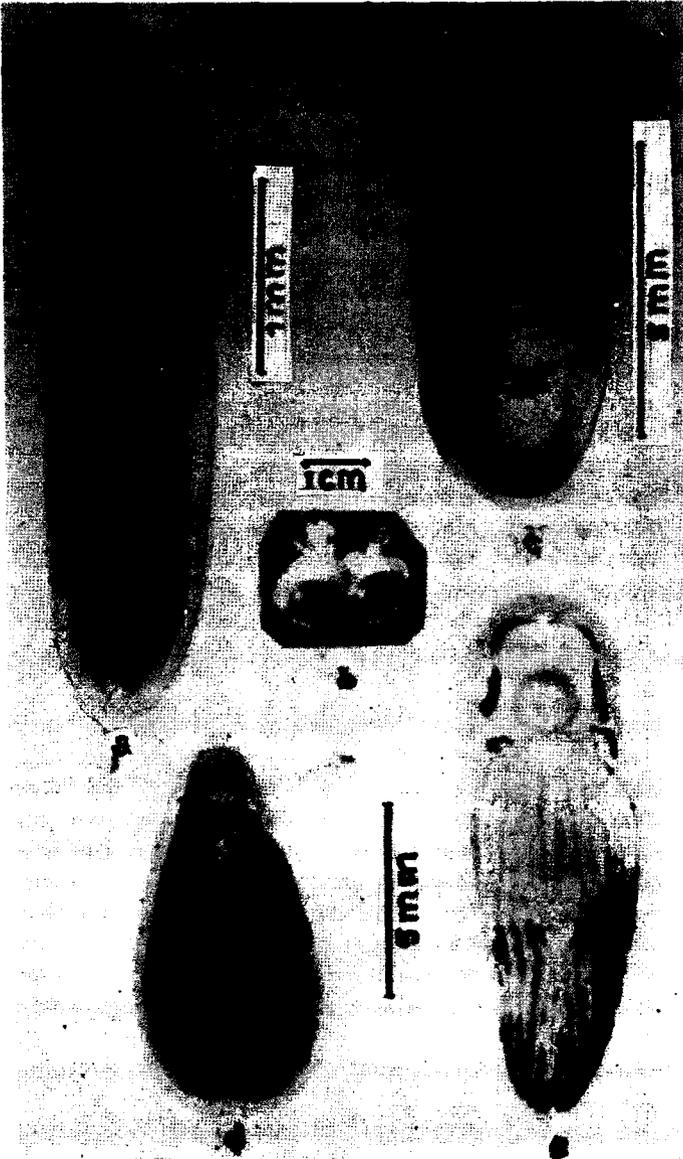


Fig. 7. a. *Clinostomoides brieni* Dollfus, 1950 syn. *Clinostomum ophicephali* Tubangui and Masiluñgan, 1944 b. Clinostomid metacercaria encysted in eyes of *Ophicephalus striatus* c. metacercaria of *Clinostomum philippinensis* Velasquez, 1959 from the buccal cavity of *Nycticorax nycticorax* Linn. e. *Euclinostomum multi-caecum* Tubangui and Masiluñgan, 1935.

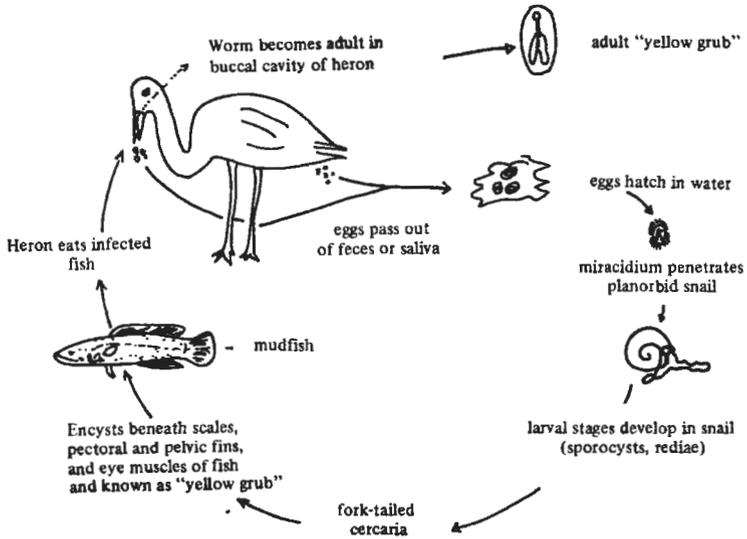


Fig. 8. Life cycle of "yellow grub" --*Cinostomum philippinensis* Velasquez, 1958.

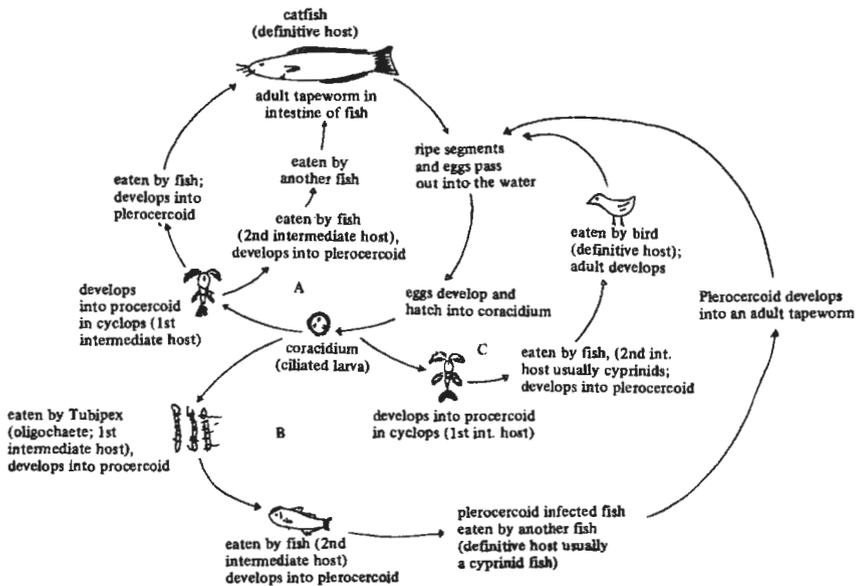


Fig. 9. Generalized life cycle of some Pseudophyllidean tapeworms found in the Philippines.

fingerlings of grass carp (*Ctenopharyngodon idellus*), big head carp (*Aristichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). *Bothriocephalus achcilonathi* have caused epizootics in carp fingerlings reared in the SEAFDEC (Southeast Asia Fisheries Development Center), Binafagonan Experimental Station. Six out of 20 fingerlings examined were heavily loaded with the "pit-viper head" or "Asian" tapeworm. *Bothriocephalus* sp. from *Glossogobius aureus* is considered new to science. The materials on hand constitute the first records of *Caryophyllideans* in this country. Importation of *Clarias batrachus* and cyprinid fishes may have resulted in transfaunation.

Fish can be either intermediate or definitive hosts for cestodes. Fig. 10 shows *Nematabramis verecundus*, a cyprinid infested with the larva of *Ligula* sp. The

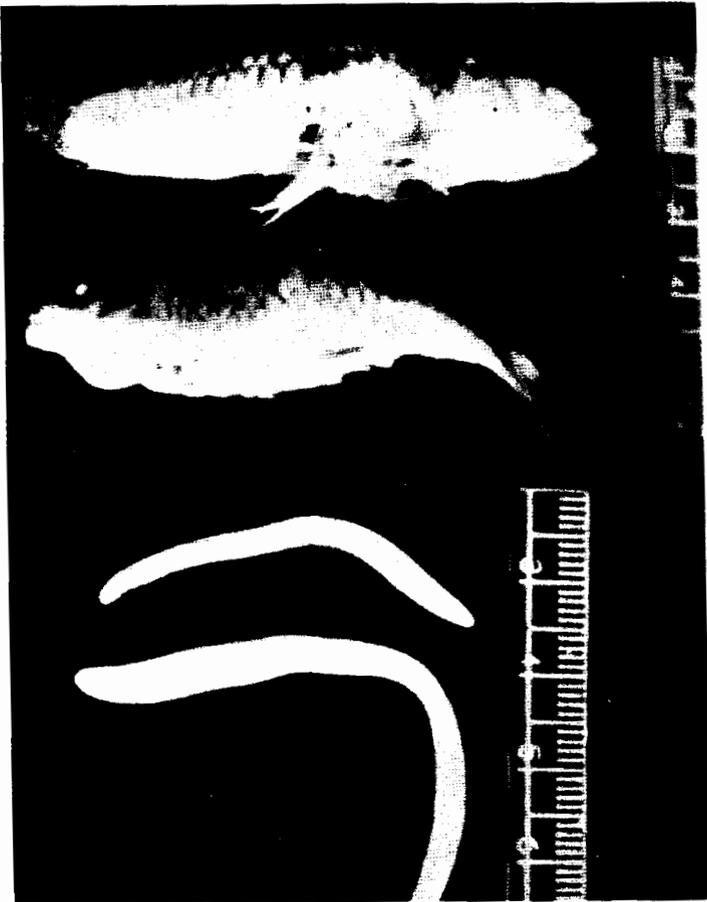


Fig. 10. *Nematabramis verecundus* (cyprinids) infected with *Ligula* sp. Note the size of the worms.

worms are undoubtedly harmful. They do not only utilize the host's food but they displace the internal organs due to their considerable size causing massive functional disturbances. The fish rapidly deteriorates and consequently dies. Heavy infestations may slow down the fish hosts which become the easy prey of piscivorous birds, the next link in the food chain. The most pathogenic and most dangerous cestode disease found in the Philippines is ligulosis.

Measures against ligulosis are limited to the removal of infested fish and killing of definitive piscivorous birds.

### Acanthocephala

The *Acanthocephala* are a clearly defined group of worms. They are cylindrical and provided with spines in the proboscis; hence are called "spiny headed worms". Sexes are separate. The females are usually somewhat larger than the males. Intermediate hosts are arthropods.

*Filisoma rizalinum* Tubangi and Masilufigan, 1946 from the intestine of the fish *Scatophagus argus* (kitang) in Malabon, Rizal and *Neoechinorhynchus octonucleatus* Tubangui, 1933 from the intestine of freshwater fish, *Therapon argenteus* (ayufigin) in Los Baños, Laguna and *Diplosetis amphacanthi* Tubangui and Masilufigan, 1937 in the intestine of fish (*Amphacanthus oramin*) in Mucielagos Bay, Mindanao are the only 3 representatives of the group in Tubangui's (1947) checklist.

The Acanthocephalan life history may be divided into four distinct phases: (1) within the egg, the hooked *acanthor* is formed; (2) upon ingestion of a suitable intermediate host, it emerges with the gut, loses its larval hooks and becomes *acanthella*; (3) it progressively develops into a juvenile (an *infective* stage); (4) when the infected intermediate host is eaten by an appropriate definitive host or final host, the *juvenile* is liberated and immediately attaches itself by its proboscis to the intestinal mucosa of the host and becomes an adult. (Fig. 11). A final host may also become a potential intermediate host.

Benthic crustaceans (Gammarus, Asellus, etc.) most frequently serve as intermediate hosts and less often, aquatic insects.

Numerous worms were found in the intestinal walls of *sabalo* (spawner, *Chanos chanos*) from Nasugbu, Batangas and BFAR Station in Mindoro Island. Ulcerations of the intestinal wall was evident with the proboscis of the worm securely attached (Fig. 12). The *sabalo* could have acquired the *Acanthocephalus* sp. infection in the open sea by eating infected arthropod intermediate hosts. When spawners are reared in tanks, filtered water from the sea is recommended. In cages in coastal areas, infection is possible. Treatment may not solve the problem due to possibility or reinfection.

Bañgas from fishponds have been found negative to *acanthocephalosis*. Marine or freshwater fishes when reared in brackishwater ponds usually lose some of their infections.

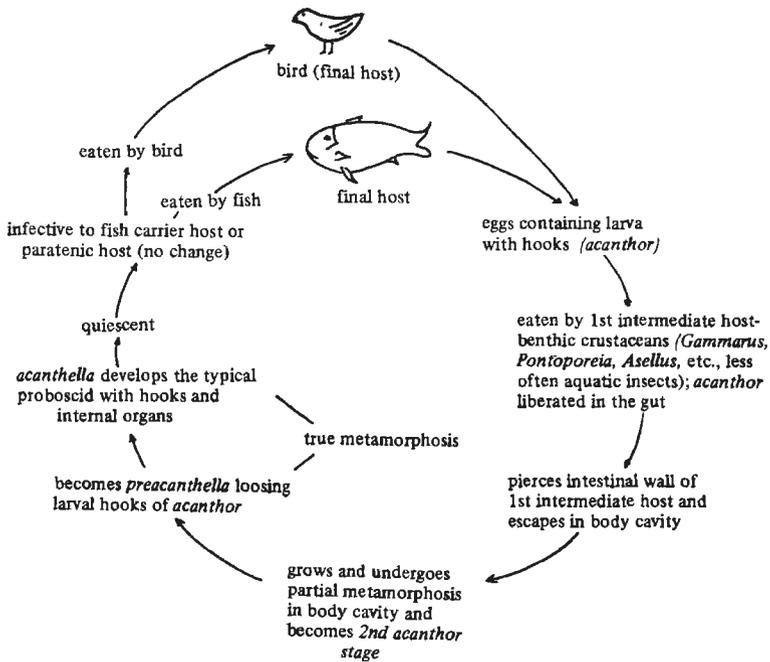


Fig. 11. Acanthocephalid life cycle (adapted from Petrochenko, 1956)

## Nematoda

Cylindrical, unsegmented roundworms belong to this group of helminths. Sexes are separate and dimorphic. Some are oviparous and others, viviparous. Development is completed in fishes or one of their developmental stages in them. Eggs or larvae are passed into the water with the feces of infected fish. As intermediate hosts, various invertebrates become infected by eating them and in turn the fish acquire the infection by feeding on the infected invertebrates or carrier (paratenic) hosts.

As of 1947 (Table 1), only 2 nematodes of fishes have been known, namely *Metabronema caranxi* Tubangui and Masilūngan, 1937 from the abdominal cavity of *Caranx speciosus* in San Narciso, Tayabas and *Gnathostoma spinigerum* encysted in the visceral linings and muscles of *Glossogobius giurus* (biyang puti), *Ophicephalus striatus* (dalag) and *Therapon argenteus* (ayūñgin).

*Cyclops (Eucyclops) serrulatus* and *C. (Microcyclops) bicolor* were found to be the crustacean intermediate hosts of *Gnathostoma spinigerum* in the Philippines

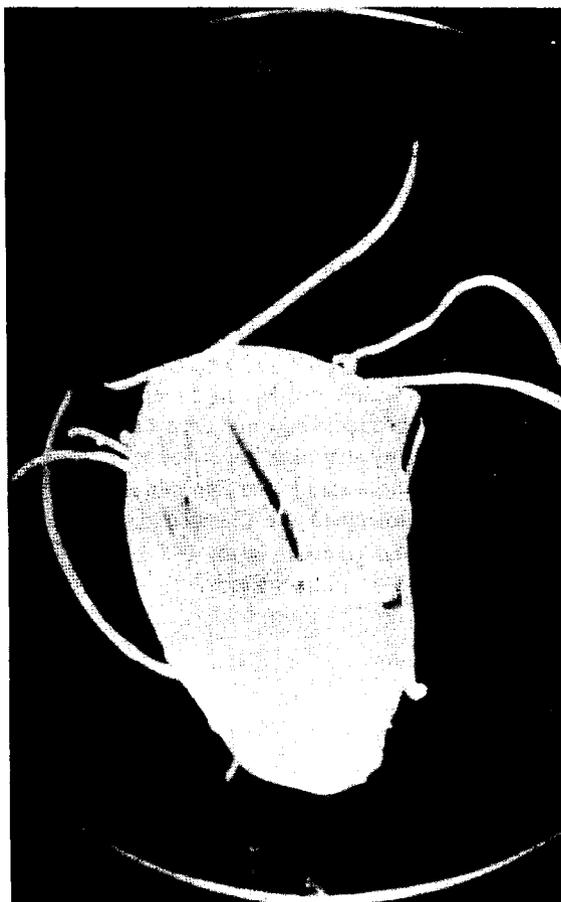


Fig. 12. *Acanthocephalus* sp. attached to the intestinal wall of *Chanos chanos* (*sabalo*) spawner.

(Refuerzo and Garcia, 1938). When eaten by freshwater fish, they become infective to piscivorous mammals including man. Swelling, edema or creeping eruptions are manifestations of gnathostomiasis infection in humans. Yogore and Juliano (1951) have reported lung involvement in a Chinese in the Philippines.

Velasquez (1966) found 6 genera of Anisakinae in 47 species of marine fishes some of which are of aquaculture potential (Table 11). The Anisakinae have been reported to cause lethal intestinal eosinophilic syndrome in Holland (van Thiel *et al.*, 1960). Several human lethal cases were reported in Japan (1967). Adults live in the intestines of whales, dolphins and other marine animals. Eggs of the parasite pass out of the definitive host, develop in the water and are eaten by invertebrates,

which when eaten by fish acquire intestinal infection. The worms from the intestine of dead fish penetrate the intestinal wall and migrate to the muscles. Man may accidentally acquire the infection by ingesting raw or not well cooked infected fish. Hence, it is recommended that fish caught should be degutted immediately before transport to the market to prevent the migration of the worms. Precautionary measures should be observed.

Velasquez (1980) reported 5 species of Camallanidae in the intestines of Philippine fishes. They are sexually dimorphic, viviparous worms whose habits are similar to the hookworm which are persistently attached to the intestinal wall.

The life cycle of *Camallanus ophicephali* was experimentally demonstrated by Calhoun (1981) in this country (Fig. 13) *Cyclops (Microcyclops) varicans* and *Mesocyclops leuckarti* served as the intermediate hosts. At temperatures 27-31°C, first stage larvae that have been ingested by the copepods develop into the second stage in 2 to 3 days and the third stage in 5 to 6 days. *Ophicephalus striatus* become infected with *C. ophicephali* by ingesting copepods with third stage larvae. A second intermediate host is not necessary in the completion of the life cycle.

Contributions to the biology of the Camallanidae in this country are those of Somsak (1982) and Vasquez (master thesis, 1982) on *Camallanus ophicephali* and Josef (master thesis, 1985) on *Procamallanus clarius*, a new record from the intestine of *Clarias batrachus* in the Philippines.

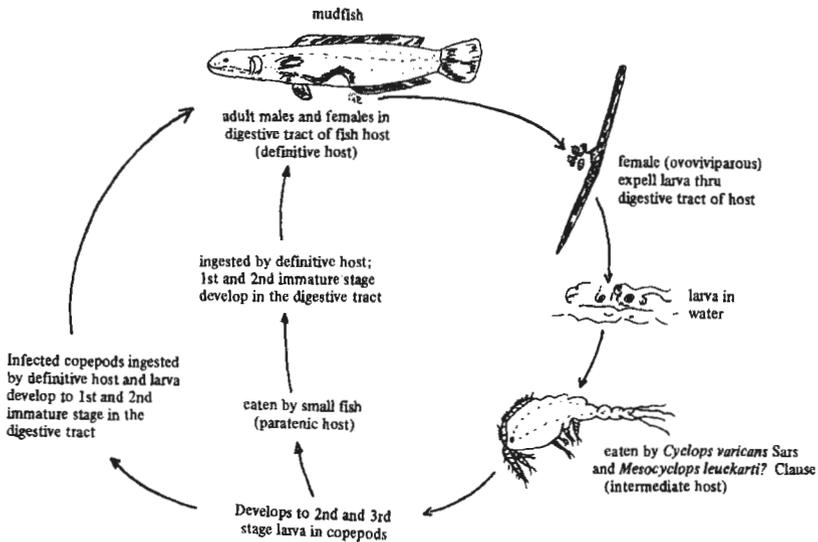


Fig. 13. Life cycle of *Camallanus ophicephali* Pearse, 1933 (after Calhoun, 1981).

Table 11. List of Philippine marine fishes harboring Anisakinae (Heterocheilidae: Nematoda)

Name of Fish	Common Name (Tagalog)	Locality	Anisakinae*						
			1	2	3	4	5	6	7
1. <i>Alectis</i> sp.	“pampanong puti”	Manila Bay			x				
2. <i>Apogon ellioti</i> Day	“langaray”	Iloilo, Visayas Is.			x				
3. <i>Caranx armatus</i> (Forskål)	“talakitok”	Navotas, Rizal			x				
4. <i>Caranx</i> sp.	“talakitok”	Malabon, Rizal		x	x		x		
5. <i>Caesio lunaris</i> Cuvier	“dalagang bukid”	Malabon, Rizal			x		x		
6. <i>Decapterus</i> sp.	“galunggong”	Quiapo market		x					x
		Malabon, Rizal		x					
		Navotas, Rizal		x					
		Zambales		x					
		Pasay City market		x					x
		Cavite		x	x				
7. <i>Eleutheronema tetradactylum</i> (Shaw)	“mamali”	Bocaue, Bulacan			x				
8. <i>Epinephelus</i> sp.	“lapu-lapu”	Malabon, Rizal			x				
9. <i>Euthynnus yaito</i> Kishinouye	“tulingan”	Quiapo market		x	x				
		Manila Bay			x				
10. <i>Gerris filamentosus</i> Cuvier	“malakapas”	Navotas, Rizal			x				
11. <i>Lactarius lactarius</i> (Bloch & Schneider)	“pilyan”	Quiapo market			x		x		
12. <i>Leiognathus equulus</i> (Forskål)	“sapsap”	Malabon, Rizal			x				
13. <i>Leiognathus</i> sp.	“tambong”	Pto. Galera, Or. Mindoro			x				
14. <i>Leiognathus</i> sp.	“sapsap”	Malabon, Rizal			x		x		
		Quiapo market			x				
15. <i>Lutianus dodecanthus</i> Bleeker	“maya-maya”	Manila Bay			x		x		
16. <i>Lutianus malabaricus</i> (Bloch & Schneider)	“maya-maya”	Malabon, Rizal			x		x		

Table 11. continued

Name of Fish	Common Name (Tagalog)	Locality	Anisakinae*						
			1	2	3	4	5	6	7
17. <i>Lutjanus vitta</i> Quoy & Gaimard	"maya-maya"	Quiapo market			x				
	"maya-maya"	Pto. Galera, Or. Mindoro				x			
	"bangbangin"	Manila Bay		x	x		x		
18. <i>Megalaspis cordyla</i> (Linnaeus)	"oriles"	Obando, Bulacan		x	x				
		Navotas, Rizal					x		
		Damortis, La Union					x		
19. <i>Mene maculata</i> (Bloch & Schneider)	"hiwas"	Manila Bay		x			x	x	
20. <i>Mollienesia latipinna</i> Le Sueur	"bubuntis"	Malabon, Rizal			x		x		
						adult ♂			
21. <i>Muraenesox cinereus</i> (Forskäl)	"pindangga"	Malabon, Rizal			x				
22. <i>Nemipterus</i> sp.	"bisugo"	Malabon, Rizal			x				
23. <i>Otolithes ruber</i> (Bloch & Schneider)	"alakaak"	Malabon, Rizal						x	
24. <i>Oxyurichthys microlepis</i> (Bleeker)	"talimusak"	Malabon, Rizal			x				x
25. <i>Pinjalo pinjalo</i> (Bleeker)	"sulid"	Manila Bay			x		x		
26. <i>Priacanthus tayenus</i> Richardson	"bisugong china"	Iloilo, Visayas Is.			x	x	x		
		Malabon, Rizal			x				
27. <i>Psettodes erumet</i> (Bloch & Schneider)	"dapa"	Manila Bay			x		x		
28. <i>Pseudoscianea aneus</i> Bloch	"alakaak"	Navotas, Rizal			x				
29. <i>Rastrelliger brachysomus</i> (Bleeker)	"hasa-hasa"	Iloilo, Visayas Is.			x		x	x	x

Table 11. continued

Name of Fish	Common Name (Tagalog)	Locality	Anisakinae*						
			1	2	3	4	5	6	7
30. <i>Rastrelliger chrysozonus</i> Ruppell	"hasa-hasa" "alumahan"	Iloilo, Visayas Is.			x		x		
31. <i>Sardinella longiceps</i> Cuvier & Valenciennes	"tamban" "turay"	Malabon market Quiapo market Nasugbo, Batangas			x x x				
32. <i>Sardinella perforata</i> (Cantor)	"tambang lapad"	Malabon, Rizal Damortis, La Union		x	x		x		
33. <i>Sardinella sirm</i> (Walbaum)	"tonsoy"	Quiapo market					x		
34. <i>Saurida tumbil</i> Bloch	"kalaso"	Malabon, Rizal			x				
35. <i>Scatophagus argus</i> Linnaeus	"kitang"	Obando, Bulacan					x		x
36. <i>Scomberomorus commerson</i> Lacepede	"tanguingi"	Quiapo market Damortis, La Union			x		x		x
37. <i>Selar crumenophthalmus</i> (Bloch)	"matang baka"	Nepa Q-mart Quiapo market			x x				x
38. <i>Selaroides leptolepis</i> Cuvier	"salay-salay"	Navotas, Rizal		x	x		x		x
39. <i>Sparus berda</i> Forskål	"bakoko"	Quiapo market	x		x		x		
40. <i>Sphyaena langsar</i> Blecker	"tursilyo"	Quiapo market Malabon Rizal					x		
41. <i>Stolephorus</i> sp.	"dilis"	Malabon, Rizal Parañaque, Rizal	x	x x					
42. <i>Synaptura sorsogonensis</i> Evermann & Seale	"dapa"	Manila Bay			x				
43. <i>Teuthis</i> sp.	"samaral"	Pto. Galera, Or. Mindoro Quiapo market			x x		x		
44. <i>Therapon jarbua</i> (Forskål)	"bagaong"	Malabon, Rizal	adult ♂ & ♀		x ♂	&	♀		

Table 11. continued

Name of Fish	Common Name (Tagalog)	Locality	Anisakinae*						
			1	2	3	4	5	6	7
45. <i>Trichiurus haumela</i> (Forskäl)	"pingka"	Manila Bay			x				
46. _____	"boloan"	Dagat, Malabon, Rizal		x	x		x		
47. _____	"igat"	Obando, Bulacan			x		x		

## Types of larvae:

- |                 |                                 |
|-----------------|---------------------------------|
| 1 - Amplicaecum | 5 - Porrocaecum                 |
| 2 - Anisakis    | 6 - Raphidascaria               |
| 3 - Contraecum  | 7 - Ascarid larvae (very young) |
| 4 - Multicaecum |                                 |

## Recommendations:

1. Remove entrails immediately after collection and before freezing.
2. Cook fish well before consumption.
3. For those interested in the study of incidence, seasonal variation of worm populations in fishes, accurate diagnosis is necessary.

## Crustacea

The crustacean parasites of aquaculture fishes in the Philippines are represented by species of Copepoda, Branchiura and Isopoda.

### Copepoda

Representatives of 4 genera of this group are found in cultured fishes in South-east Asia (Kabata, 1985). Recorded in the Philippines are *Lernaea cyprinicea* Linn., 1758, *Caligus patulus* Wilson, 1937, *Argulus* sp., *Argulus indicus* Weber, 1982, and *Ergasilus philippinensis* Velasquez, 1951.

*Lernaea cyprinicea*. The females become attached to the host by deeply burrowing into the tissues with the highly modified anchor-shaped anterior end. The exposed protruding body bears two egg sacs at its posterior end. The parasite undergoes several microscopic developmental stages usually in the gills of the fish other than where it develops to maturity. Males are free-living. Infected fingerlings reared in the Himlayan Filipino fishponds and in the fish pens of Laguna de Bay were referred to the author for diagnosis. The wormlike copepod protruded from the nostrils and skin of the infected fish usually at the base of the fins. (Fig. 14a). The visible part is cylindrical, whitish, often with two eggs extending from its posterior end. When carefully dissected out of the fish, the modified head is anchor like, giving it the name "anchor worm". This parasite goes through developmental stages from fish to fish resulting in considerable damage to the host. Mass infestation resulted in great economic loss.

Salt solution baths of about 3-5% concentration proved effective. However, the adults were difficult to kill, hence dessication and liming of infected ponds were recommended before restocking of healthy fish. No recommendations were given for those in fish pens in Laguna de Bay. Control of epizootics in a big lake is a futile case.

*Caligus patulus*. The diagnosis requested by the SEAFDEC (Southeast Asia Development Center) Aquaculture Department in Pandan, Antique for this copepod attached to the skin, fins and gills of tank-reared milkfish brood stock (sabalo) at the time was *Caligus* sp. On this generic identification, Laviña (1978) reported 0.25 ppm Neguvon bath for 12-24 hours effective in removing them. Repeated treatments at intervals of several weeks were found necessary as the copepod infestation recurred. Jones (1980) redescribed *Caligus patulus* Wilson, 1937 (Fig. 14b) based on specimens sent to him from the Philippines.

All members of the family Caligidae are parasites. They cling to their hosts' surface with their flat bodies somewhat like a large adhesive disc aided by the prehensile appendages. They are capable of movement over the host surface.

The first larval stages and the final adult stage are free-swimming. The adults occur free in plankton. The second pair of antennae are provided with claws and have two sucker-like organs located close to the anterior end of the cephalothorax. The *nauplii* are positively phototropic and swim up close to the surface of the water.

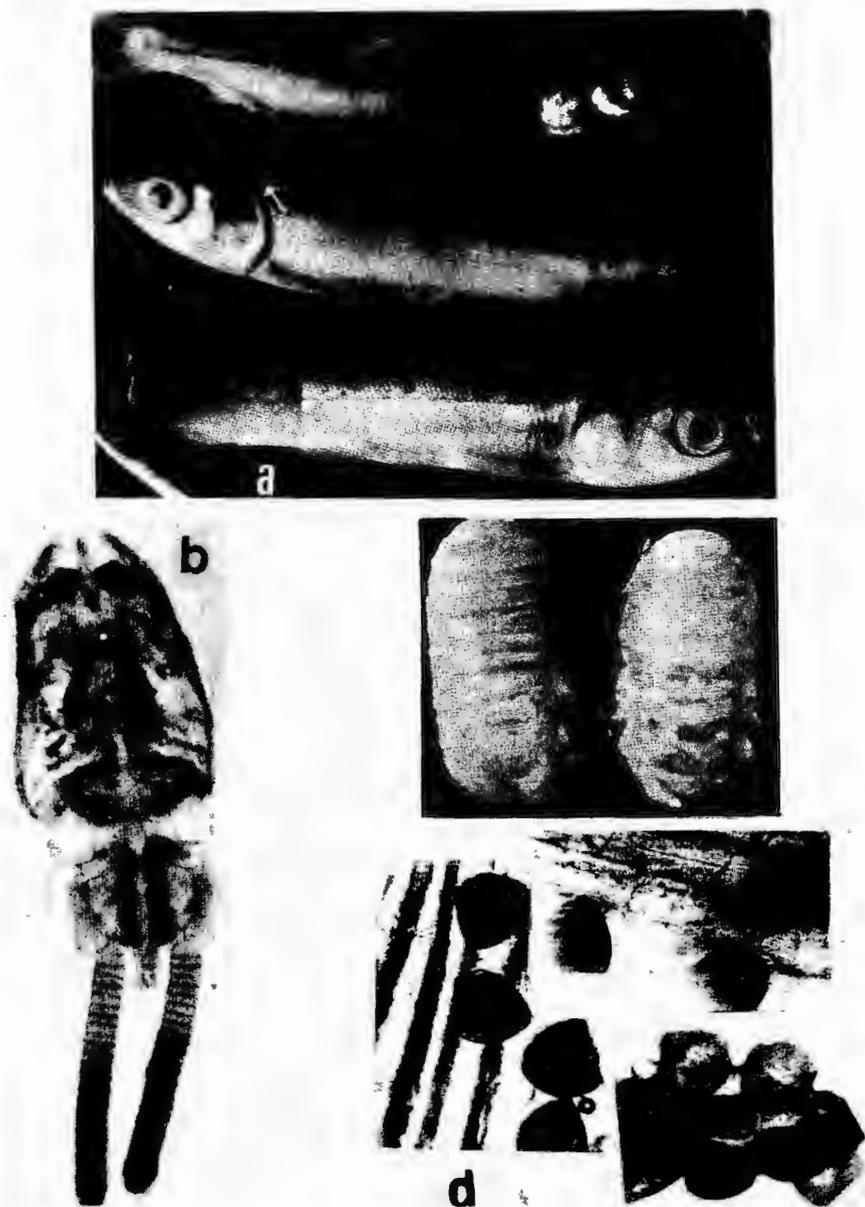


Fig. 14. a. *Lernaea cyprinacea* Linn. attached to different parts of *Chanos chanos* fingerlings. b. *Caligus patulus* Wilson from *Chanos chanos* spawners reared in tanks. c. *Alitropus typus* syn. *Rocinela typus* d. Glochidia of *Cristaria plicata* in tissues of experimentally infected *Clarias batrachus*.

The final metamorphosis stage, however, becomes negatively phototropic and finds its host at the lower level of the water near the bottom. The characteristic *copepodid* stage in caligids is generally known as *chalmus*. It is attached to the host, usually to a fin or scale, by a long filament that is formed by the hardening of the secretion derived from a gland located in front of the eyes. The larvae undergo several molts while remaining attached in this manner, each molt, a stage closer to the adult. When the adult male emerges from its final molt, it breaks off the thread and immediately searches for a female. The latter remains until fertilized and only then does it break away to lead a planktonic existence. However, confinement as in the case of the bangus (*sabalo*) in rearing tanks, the caligids from the sea being planktonic, find their way to the fish hosts and wander over their bodies sucking their food with the aid of a tubular mouth part. Caligid adults never stay far from their hosts. In the samples sent to me, some males have been found still hugging the females. At the time, the source of water of the *sabalo* experimental tanks in Pandan, Antique was directly from the sea without adequate filters. Concentration of the planktonic caligids resulted in the infestation of the *sabalo* and death of a number of them. Recommendations to provide adequate filtration facilities were suggested.

For treatment, the author recommended the use of 1-3% formalin bath since it is less harmful to other organisms in the tank. Neguvon (Dylox) in sea water dissipates faster.

Recent findings indicate that both Neguvon bath and food additives left residues in tissues (Brandal and Egidus, 1977 cited by Kabata, 1985). Precautionary measures should be observed in their use. Prophylactic measures, treatment, prevention and control should be in accordance with the existing ecological conditions in the locality. Chemotherapy of fish for human and farm animal consumption should be employed in accordance with Food and Drug rules and regulations.

*Argulus* sp. commonly called "fish louse", is dorso-ventrally flattened with ventral discs adapted for attachment to the skin and scales of fish. Infected fish are injured by the piercing mouth parts. The argulids are sexually dimorphic and both sexes are parasitic. Eggs are borne in sacs at the posterior end of the female. The eggs drop off in the water and are attached to stones and other suitable substrate. When developed, they hatch under favorable conditions and become infective to fish. They have been found in *Cyprinus carpio*, *Tilapia* sp., mullets and eel. Mortalities have been reported in infected Japanese ornamental carps (Po *et al.*, 1982). *Argulus indicus* occurs in *Ophicephalus striatus*, *Carassius* sp.

*Ergasilus philippinensis*. This is the only Philippine representative of *Ergasilus* in the Philippines found by Velasquez (1951) in the gills of *Glossogobius giurus* from Laguna de Bay. The genus *Ergasilus* infests the gills of many freshwater fishes. They are sexually dimorphic, the females being parasitic and the males free-living. Gills infected in great numbers are often with haemorrhagic spots and at times greatly damaged. Eggs hatched into nauplii within a few hours. Further development was not observed.

## Isopoda

*Rocinela typus*, Fig. 14c now considered *Alitropus typus* by experts is a vicious killer. It kills not only the fry and fingerlings but also fish of marketable size. Observations under laboratory conditions showed that they attack the fish, traumatise, and eventually kill them. The mass infestation of *Chanos chanos* resulting in great mortalities in the Iloilo fishponds was referred to the author by former Dean Abelarde of the U. P. College in Iloilo (1968).

Direct life cycle and fast multiplication enhanced the intensity and incidence of infection. It was recommended that: (1) all infected fish be removed from the ponds (2) survivors and non-infected ones be placed in clean ponds, (3) all ponds with infected fish be dried and limed for several weeks before restocking.

*Epipenaeon ingens* Nobili of the family Bopyridae is now recorded infecting the shrimp, *Penaeus semisulcatus* in this country. Host-parasite relationships have been studied (master thesis Palisoc, 1982).

## Annelida

Leeches are flattened, segmented worms with suckers at each end. The mouth is provided with a rasping organ which is adapted for blood sucking. They are known to transmit a flagellate, *Trypanoplasma cyprini* to carp. Instances of *Piscicola* sp. infections have been found in *Ophicephalus striatus* (unpublished). No mass mortalities have been recorded in this country.

## Mollusca

Glochidia are larvae of some freshwater clams attached to the gills and skin of fish where they remain from 10 to 20 days. The fish tissue grows over the larvae producing a cyst. When in great numbers considerable damage occurs in the infected fish. Fig. 14d shows glochidia of *Cristaria plicata* encysted in the skin and other tissues of experimentally infected *Clarias batrachus*. Polyculture of fish and this bivalve must have to be reexamined for detrimental effects of the glochidia on infected fish.

## Perspectives

Accelerated research efforts in the field of fish parasitology are imperative, considering that disease is one of the serious factors in fish health management in the aquaculture industry.

Future regulations to restrict introduction of disease-carrying fish will minimize problems created by transfaunation resulting from importation of fish intra-

nationally or internationally. We should look forward to the development of disease-free fish by better design of facilities and sanitation. Less resistance to parasitic diseases may be the outcome of improper handling of transported fry causing stress or nutritionally unsound feeds that are not properly compounded and stored.

Fish parasites and diseases do not seem to be a priority in the program of aquaculture, despite the problems that arise sporadically when they do occur.

An accessible Center of Fish Parasites and Diseases with adequate laboratory and library facilities, not to mention qualified personnel, is now envisioned. Such a center may soon be a reality with financial assistance by IDRC (International Development Research Center of Canada) and cooperation of the BFAR. Dr. J. Arthur, a protozoologist, is now in charge of the project. The agency may also serve as a Training Center in Fish Parasitology. Subcenters in areas where there are extensive aquaculture programs can monitor local problems.

Fisheries research in all its aspects should be intensified towards a successful cultivation of our fishes and the effective exploitation of the resources of our waters.

### Acknowledgments

No single individual can claim to solve all problems in fish parasitology. The cooperation of people interested in the discipline cannot be overlooked.

This assessment will be incomplete if it fails to mention the works of the author's masteral and doctoral thesis students in Zoology who have also contributed to the knowledge now gained. Their contributions are listed in the references.

The PCARRD's (Philippine Council for Agriculture and Resources Research and Development) role in providing funds and other incentives is recognized.

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